TYMPANOSCLEROSIS

AN EXPERIMENTAL AND CLINICAL STUDY

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Proefschrift Katholieke Universiteit Nijmegen. - Met lit. opg. - Met samenvatting in het Nederlands
ISBN 90-9009719-8
Trefw.: tympanosclerosis

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AN EXPERIMENTAL AND CLINICAL STUDY

Een wetenschappelijke proeve
op het gebied van
de Medische Wetenschappen

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Katholieke Universiteit Nijmegen
volgens besluit van het College van Decanen
in het openbaar te verdedigen
op donderdag 12 september 1996
des namiddags om 1 30 precies

door

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geboren 24 november 1954 te Rotterdam

Druk Grafisch Bedrijf van Lieie B V , Emmen

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Publication of this thesis was generously supported by:

Asta Medica B.V., Astra Pharmaceutica B.V., Entermed B.V., Glaxo Wellcome B.V., GN Danavox Nederland B.V., HAL Allergenen B.V., Mediprof B.V., Ooms Allergie B.V., Pfizer B.V., Rockmed B.V., Veenhuis Medical Audio B.V.

Dedicated to the memory
of Gordon D.L. Smyth

CONTENTS

GENERAL INTRODUCTION

Tympanosclerosis is a middle ear disease, affecting both the tympanic membrane and the middle ear mucosa. It is characterized by accumulation of abnormal collagen, hyalin degeneration and calcification. It is a frequent sequela of chronic otitis media and has also been associated with trauma to the tympanic membrane.
Although symptoms and signs of tympanosclerosis may be minimal in some cases, extensive tympanosclerotic plaques may create a serious impedement with regard to hearing in other cases.
The first comprehensive descriptions date back to the 19th century, but it wasn't until the second half of this century that interest in the disease was re-initiated, in connection with the introduction of the operating microscope.
This incited many clinical and epidemiological studies into tympanosclerosis, in which a strong correlation of the disease with chronic otitis media was shown. After the introduction of the tympanostomy tube as a treatment for chronic otitis media with effusion in children, it became apparent that direct trauma to the tympanic membrane through insertion of these tubes showed significant enhanced formation of tympanosclerosis as well.
To gain further insight into the pathogenesis of the disease, also light- and electronmicroscopical studies of the morphological features have been performed. Although these have added considerably to our knowledge of the affliction, a definite pathogenetical pathway has still not been established. In animal models, attempts have been made to induce tympanosclerosis, but these have so far been unsatisfactory. In the course of an earlier study in the histological laboratory of the department of otorhinolaryngology of the University of Nijmegen, an accidental finding led to a growing interest in the pathogenesis of tympanosclerosis. It was found, that germ-free rats, in which the eustachian tube was blocked by a surgical procedure, developed a sterile effusion in the middle ear and also extensive signs of tympanosclerosis. This remarkable finding opened a new possibility for further studies into the pathogenesis of tympanosclerosis.
Site and extent of the sclerotic lesions may differ according to particular etiological events, but the end product has an unvarying histopathology: an accumulation of abnormal collagen and hyalinisation and calcification. The process most frequently occurs in the lamina propria of the tympanic membrane, but may also affect the lamina propria of the mucosa that covers the auditory ossicles and lines the bony wall of the middle ear. The lesions may vary from small, nearly asymptomatic plaques in the tympanic membrane or at other sites of the tympanum to large aggregations which may render the tympanic membrane stiff and inflexible and even envelop ossicles, with subsequent ankylosis, inducing moderate to severe conductive hearing loss.
Management of tympanosclerosis depends largely on site and extent of the lesions. Solitary plaques situated in the tympanic membrane usually have no clinical significance in terms of hearing loss and, if so, they are left in place as not to unduly disrupt the eardrum in an attempt to excision. With regard to tympanic cavity tympanosclerosis, management is debated on, but it has become clear that surgical procedures especially on the stapes carry great risk for iatrogenic sensorineural hearing loss in these cases.

The purpose of this study was three-fold First to critically analyze the different theories presented in the literature with respect to the pathogenesis and the clinical aspects of tympanosclerosis Secondly, to address a number of unsolved issues regarding the behaviour of the lamina propria in the development of tympanosclerosis by means of a histopathological study in an animal model, developed in our laboratory The experimental model used, allowed for investigation of the influence of different kinds of middle ear effusions as well as the effect of direct surgical trauma on the behaviour of the lamina propria in relation to the development of tympanosclerosis Thirdly, to analyze the long term results of tympanoplasties performed on patients at our department with tympanosclerosis of the tympanic membrane with reference to the closure of the tympanic membrane and hearing

CHAPTER I

ANALYSIS OF CURRENT CONCEPTS OF TYMPANOSCLEROSIS

E.W.J. Wielinga & A.G. Kerr

Clin Otolaryngol 1993; 18: 341-349

Tympanosclerosis is an abnormal condition of the middle ear characterized by calcareous deposits in the tympanic membrane, tympanic cavity and occasionally in the mastoid

The first description of tympanosclerosis dates back to Cassebohm who described "chalky layers" in the eardrum in 1734 [1] In this respect the term "sclerosis" was first used in 1873 by von Troltsch[2] who gave a comprehensive description of a dry catarrh or "sklerosis" of the mucous membrane of the middle ear, relating the condition to chronic ear disease In other early descriptions of middle ear infections, sclerosis of the tympanic mucosa was noted to be a frequently occurring sequela, which rendered the mucosa stiff and inflexible [1 2 3] It was evident that all soft tissues in the tympanic cavity could undergo these sclerotic changes because they were found not only in the tympanic mucosa but also in the tympanic membrane, inter-ossicular joints, ossicular ligaments, annular ligament, stapedial tendon and the tendon of the tensor tympani muscle

Recognition of the clinical implications of the disease became only fully apparent in the middle of this century, in connection with the rapidly growing interest in reconstructive middle ear surgery This renewed interest culminated in the publication of a paper by Zollner and Beck entitled "Die Paukensklerose" in 1955 [4] Zollner translated "Paukensklerose" into "tympanosclerosis" and introduced this term into the English literature [5]

Pathogenesis, light- and electron-microscopical characteristics and surgical management were extensively studied and investigators even succeeded in inducing tympanosclerosis experimentally Despite these studies, which have considerably improved our knowledge of the character of the disease, the aetiology and pathogenesis are still obscure, in addition there is still controversy concerning its surgical management Some consider tympanosclerosis "the least amenable of the diseases of the middle ear which we attempt to treat surgically",[6] while others claimed it to be safe for surgical intervention in experienced hands [7]

1. PATHOGENESIS

The uncertainty which exists concerning the pathology of tympanosclerosis is well illustrated by the variety of equivalent expressions that are used in the literature to describe the condition, such as "chronic catarrhal otitis media",[8] "sclerosing mucositis",[9] "a special result of a healed inflammation"[10] and "a peculiar form of scar tissue" [11]

It is generally assumed that multiple acute or chronic inflammatory processes either of purulent or serous nature, affecting the middle ear are the most important aetiological events,[12 13] and tympanosclerosis is therefore categorized as one of the forms of non-otosclerotic post-inflammatory fixation of the middle ear ossicles [14 15] Some even consider only one episode of severe necrotizing otitis media sufficient to initiate the process, in view of its frequent occurrence noted after one episode of scarlet fever [16 17]

Another important aetiological factor is tissue trauma, which is substantiated by the frequent occurrence of tympanosclerosis after insertion of ventilation tubes Nevertheless the exact aetiology and pathogenesis of the disease is still not completely clear

In the many histological studies that have been performed it has been well established that the pathological changes of tympanosclerosis are situated in the lamina propria, which is the connective tissue component of tympanic membrane and mucosa. *(Figure 1).*[18,19,20]

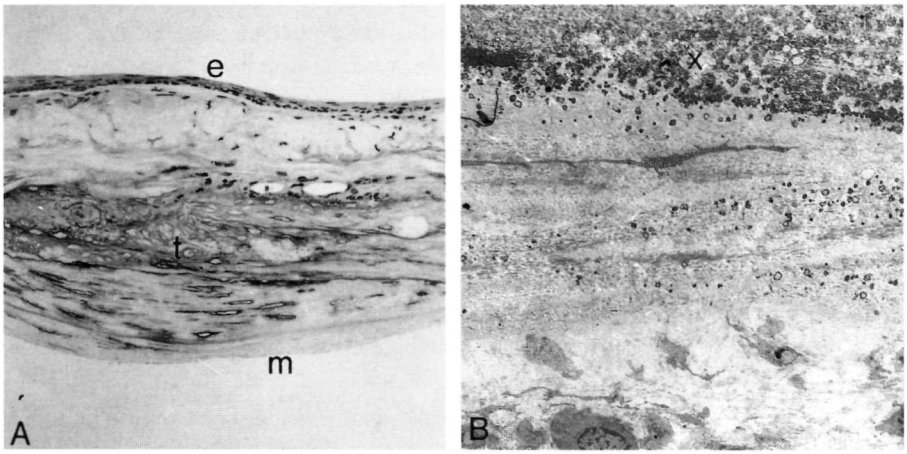

Figure 1. Lightmicrograph (A) and electronmicrograph (B) of tympanosclerosis in tympanic membrane. The membrane is thickened and shows severe structural disarrangement. Tympanosclerotic lesions (t) showing scattered calcifications in the lamina propria. Electronmicrograph in B shows numerous calcareous deposits in the dense collagenous tissue. e: epidermis; m: middle ear epithelium.
A: Toluidin blue staining; magnif. x 180; B: magnif. x 1800.

1.1 The role of inflammation

Gibb[16] assumed two stages in the process: an initial stage of active collagen production with a cellular submucosal inflammatory reaction and a deeper area with fibroblastic activity, with additional layers of collagen, hyalinization, calcification and even bone creation, and a subsequent non-active stage characterized by the existence of mature collagen masses.
In the majority of studies that were made on the development however, three stages are recognized. At the initial stage, inflammatory processes lead to damage to collagen fibres. Some emphasized the role of inflammatory exudate, trapped and stagnating in the many recesses in the middle ear, in which organization takes place.[15,16,19,21,22] Others stated that the first step in the process is the formation of granulation tissue: during acute inflammation, the tympanic mucosa and submucosal tissue become oedematous and infiltrated with many inflammatory cells; with repeated acute or prolonged chronic inflammation, granulation tissue may be formed.[12,13,18,23,24-25] This phase is generally considered reversible.
The second stage is the reparative phase, characterized by fibroblast invasion.[13,23] This results in excessive collagenesis and hyalinization, as a result of which fibres become indistinct, fusing into an homogeneous mass.[22] Clinically this tissue appears as smooth, white, slightly raised areas of dense tissue having a cartilaginous or rubbery texture. It is possible to peel off this tissue, at times resembling onion layers.[16,23]

Most authors consider the process now to be irreversible and in the third and final stage calcification and occasionally ossification may occur The appearance of calcification in the collagen matrix has been explained in various ways Igarashi et al [12] stated that in a hyalinized or necrotic tympanic mucosa dystrophic calcification is a common feature, quoting Boyd[26] who stated that there is a tendency for the development of calcification in any dead tissue Others suggested four stages of mineralization, the first stage characterized by the appearance of matrix vesicles, the second by the formation of calcospherules, the third by progressive mineralization and in the final stage large masses of calcified collagen are formed [24 27] Scanning electron-microscopic studies showed this calcified tissue to contain fissures with dense irregular collagen fibres in between housing calcospherules [28]

Schiff et al [29] attempted to prove a possible involvement of auto-immunity in the pathogenesis of tympanosclerosis Their proof was based on an animal experiment in which injection of a tympanic membrane-antibody into a previously sensitised gui nea-pig, produced the immune complex at the injured site It was argued that bacterial infections producing lytic enzymes damaged the ground-substance through oedema and subsequent stretching, thus presensitizing the connective tissue layers The tissue would respond with considerable scarring in the event of ensuing ear infections and in these scars calcium deposits might occur In analyses of human tympanosclerotic eardrums, however, only small amounts of fibrinogen and complement and no antibodies were found [30]

Another proposed cause was otitis media with effusion (OME) Møller[20] described degeneration of the fibrous layer due to oedema and inflammation in the submucosa in the course of OME Others ascribed the development of tympanosclerotic lesions to the traumatic effect of eardrum retraction in OME, with subsequent damage to the fibrous layer [31 32]

1.2 The role of tissue trauma

The noxious challenge which sets off the degenerative changes in the lamina propria can also be of non-inflammatory nature and the importance of tissue trauma has been widely recognized as an important aetiological factor [31 33 36] Myringotomy with or without insertion of ventilation tubes in the eardrum for instance, is associated with an increase in the incidence of tympanosclerosis of the tympanic membrane

2. CLINICAL FINDINGS

Over the years there has been considerable discussion as to whether tympanosclerosis found in the tympanic membrane is the same disease entity as tympanosclerosis found in the tympanic cavity It has been suggested that the term myringosclerosis be used when the process is confined to the tympanic membrane[37] and the term tym panosclerosis should be exclusively reserved to describe sequelae of chronic otitis affecting the ossicular chain [38 39] Morphologically, however no differentiation can be made between the two conditions

The most obvious manifestation of tympanosclerosis is in the tympanic membrane

Otoscopically, deposits present as sharply demarcated areas of whitish opaque, chalk-like material. According to some, one can often observe bloodvessels on the lateral aspect, signifying an intact epidermal layer. Plaques in the tympanic membrane usually occur only in the pars tensa, mostly situated in the anterior or posterior segments, varying in size. Following myringotomy, with or without the insertion of ventilating tubes, the pattern and extent of plaques may change.[20 40 41] Often a typical horse-shoe shaped form can be seen, extending from the antero-superior quadrant downwards around the umbo of the malleus, upwards to the postero-superior quadrant. Calcifications in the pars flaccida have been observed occasionally.[20]

2.1 Tympanic membrane tympanosclerosis

The reported incidence of tympanosclerosis confined to the tympanic membrane in chronic otitis ranged from 24% and 51%.[16 23]
The clinical importance of this calcification is dependent on size, which can vary from insignificant deposits, mostly of no clinical significance in terms of hearing loss,[15 42] to large plaques covering extensive areas of the tympanic membrane. Tos & Poulsen[43] conducted speech audiometry on chidren who were previously treated with ventilating tubes for OME and found no difference in speech reception thresholds (SRT) between ears with tympanosclerosis and ears with a healthy pars tensa. Tos & Stangerup[44] found a mean difference of maximally 1 dB at frequencies of 250, 1000 and 4000 Hz between thresholds of normal ears and ears with tympanosclerotic drums.
When large areas of the tympanic membrane are involved, however, mobility of the membrane may be impaired which will result in a mild to moderate hearing loss.[45] Mobility of the membrane may be severly reduced if the plaque is adherent to the bony annulus or the handle of the malleus or makes contact with the promontory. A large plaque may involve the whole anterior half of the tympanic membrane, fixed to the bony annulus in front and the handle of the malleus behind, causing both immobility of the tympanic membrane and fixation of the ossicular chain. The occurrence of plaques in the eardrum may indicate the presence of more extensive disease in the middle ear in patients with a history of chronic otitis. Simultaneous occurrence of tympanosclerosis in both tympanic membrane and cavity ranged from 33% to 49% in different studies.[15 46 47]
Tympanosclerosis in the tympanic membrane can also occur in OME. In a cohort study comprising 222 unselected and untreated five year old children, Tos et al.[34] found signs of tympanosclerosis in 5.4% of all ears. This percentage correlates well with findings by Schilder et al[48] who found a prevalence of 4.6% in 86 children of 7-8-years-old with persistent OME. In several other studies the prevalence in untreated OME ranged from 0% to 15.2%.[33 43 49 50 51]
A number of studies indicated that tympanosclerosis in OME showed a dynamic behaviour. In the same series of 222 children, Tos et al.[34] found an overall increase in prevalence of 8.6 % when children reached the age of 7 years but at the same time the calcification had disappeared in half of the group that initially presented with the disease. A similar finding was reported by Tos & Poulsen[43] who initially found tympanosclerosis in 49% in 527 ears followed up between 6 months and 1 year after grommet extrusion. Assessment after 3-8 years showed a prevalence of 28 %. Skin-

ner et al [51] also found evidence that tympanosclerosis can disappear after a period of time They conducted a 15- year-follow-up of patients first assessed at 5 years by Brown et al [49] and found fewer tympanosclerotic lesions after 15 years than initially reported after 5 years A possible explanation for this dynamic behaviour was given by Møller[52] who studied tympanosclerotic tympanic membranes of patients with OME by scanning electron microscopy He observed fibrocyte-like cells that seemed to invade tympanosclerotic regions in certain areas, in some cases producing fibrils

2.2 Tympanic cavity tympanosclerosis

The incidence of tympanosclerosis of the tympanic cavity ranges from 7% to 32% in ears with chronic middle ear disease [9 15 17 38 46 53]

Ears with tympanosclerosis usually have a history of multiple acute episodes of chronic otitis media To date, there are no known reports on tympanosclerosis occurring in the tympanic cavity in cases of uncomplicated OME However, because the middle ear is rarely inspected in OME it is difficult to assess the presence of such lesions in the middle ear or mastoid cavity

Although it is not known how much time it takes to develop tympanosclerosis of the tympanic cavity, it is generally assumed to be many years Gibb[16] estimated a period of 10 to 30 years for the development of clinical tympanosclerosis in the middle ear Kinney[15] noticed that 90% of the 132 patients that presented with ossicular fixation due to tympanosclerosis, were over 30 years old and had a history of ear disease of over 10 years

Often the tympanic membrane is perforated In various studies perforations were found in 86% to 100% of the ears (Table 1) These perforations are usually large and centrally situated Gibb[54] suggested a clinical classification on this basis and proposed the term "open tympanosclerosis" in cases where tympanosclerosis is present and the tympanic membrane is perforated and "closed tympanosclerosis" in cases when

Table 1. Perforation sites and percentages of dry ears

Author(s)	N	% perforation		% dry
		marginal	central	> 1 year
Sheehy & House (1962)	75	38	50	91
Zollner & Beck (1964)	71	5	60	n r
Bonneaud (1971)	85	14	73	n r
Tos & Bak-Pederson (1974)	26	n r	n r	100
Gibb (1976)	138	10	56	83
Kinney (1978)	311	21	50	87
Emmett & Shea (1978)	45	n r	n r	67
Gristwood & Venables (1982)	325	4	82	>8

the tympanic membrane is intact Another typical finding in ears with tympanoscle rosis is the absence of suppuration usually for a long period (Table 1)

Deposits in the middle ear cavity can present in various degrees and locations which eventually determine the severity of hearing loss The mildest form is called subclin ical tympanic cavity tympanosclerosis[55] when deposits are only detectable by means of light microscopical investigation of the middle ear mucosa

When clinically present, however deposits may display large masses mainly in the oval window niche, the epitympanum or on the promontory The degree of ossicular involvement can vary from a slight fixation of one ossicle to total inclusion of the entire chain in a solid mass

Tympanosclerosis must be differentiated from other middle ear lesions that cause conductive hearing loss Schuknecht[14] described three possible forms of non oto-sclerotic fixation of the middle ear ossicles fixation by fibrous tissue hyalinization of collagen and new bone growth The fixed malleus head syndrome as described by several authors, should also be kept in mind as a possible cause of fixation of the ossi-cular chain [56 60] In this syndrome an anterior epitympanic bony spur which is fre quently encountered in normal temporal bones is considered to be the cause of osse ous fixation [60]

The diagnosis of tympanosclerosis, especially in cases with an intact tympanic mem brane, may prove difficult It must be considered when a discrepancy exists between the degree of hearing loss and the clinical signs [15 38] patients suffer from a non-progressive hearing loss who have a history of past otitis media with an otherwise nor-mal tympanic membrane[16] and patients with chronic otitis media who have dry ears and have a gradually progressive conductive hearing loss [46]

With high resolution CT scanning of the middle ear it may be possible to differen tiate between fibrous tissue formation, tympanosclerosis and new bone formation [61] Tympanosclerosis has a CT appearance of unifocal or multifocal punctates or web-like calcific densities in the middle ear cavity, epitympanum or tympanic membrane Calcification of the suspensory ligaments and tendons are also relatively easy to diag nose It proves however, difficult or even impossible to differentiate tympanoscler osis of the annular ligament and/or stapes footplate from otosclerosis The CT appear-ance of fibrous tissue fixation is that of a non calcific non dependent soft-tissue debris that encases some or all of the ossicular chain New bone formation is primarily found in the epitympanum where it shows as lamellar new bone formation on existing bony structures

Hearing loss due to tympanosclerosis is usually caused by fixation of the ossicular chain Predilection sites causing fixation have a fairly constant distribution, involving either parts of the malleus and incus in the attic and/or the oval window region, affec-ting the stapes In some cases the tympanosclerotic mass envelops all three ossicles Fixation of the malleus usually includes the handle which may be involved in a pla-que of tympanosclerosis situated in the antero superior quadrant of the tympanic membrane or may be caused by sclerotic degeneration of the anterior and superior ligaments of the malleus and the spheno mandibular ligament which is inserted into the neck of the malleus after passing through the Glaserian or anterior tympanic fis-sure Occasionally deposits are massive filling the entire epitympanum [62] As is shown in Table 2, fixation of the stapes is a common finding and is almost always

Table 2. Distribution of tympanosclerosis of the ossicles

Author(s)	N	% M + I	% stapes	% M + I + S
Zollner & Beck (1964)	71	20	25	55
Faltynek & Hybasek (1964)	48	40	20	40
Gundersen (1965)	37	22	62	16
Bonneaud (1971)	65	18	32	50
Gibb (1976)	138	22	30	48
Gormley (1987)	67	—	70	30

present with a conductive hearing loss in a tympanosclerotically altered middle ear [63] In a minority of cases, discontinuity of the sound transforming mechanism is present, either as a direct consequence of preceeding episodes of otitis media[46] or caused by the sclerotic process itself, which effects a low grade bone necrosis through pressure and ischaemia [16] Occasionally reduction of tympanic air space due to extensive deposits may be a cause for hearing loss [16]

3. TYMPANOSCLEROSIS AND VENTILATION TUBES

Since the introduction of the ventilation tube in the treatment of OME by Armstrong in 1954, insertion of ventilation tubes has become the most widely performed otological procedure in the world today The value of tubes in the treatment of OME is increasingly in dispute, however, not only because most effusions will resolve spontaneously within a certain period, but also many clinical and experimental studies have established that insertion has negative side-effects on the structure of the tympanic membrane [64] The main complications that have been described are atrophy and tympanosclerosis

In different reports, the percentage of tympanosclerosis after grommets varies from 28% to 61% (Table 3) Although the observation of enhanced tympanosclerosis after grommet insertion was reported by many authors, no uniform agreement exists concerning the aetiology of this complication

Many hold the view that the degenerative changes in the pars tensa are at least partly the result of long-lasting secretory otitis media This is substantiated by a number of observations First the localization and the amount of the degenerative plaques appeared not to be related to the site of the grommet [30 33 36 41 43] Secondly, early stages of tympanosclerosis often appeared to be reversible once exudation ceased and adequate ventilation of the middle ear was obtained [51 65 66] Thirdly, it was argued that drumheads with plaques had been exposed to relatively longer periods of abnormal middle ear pressure compared with normal tympanic membranes [32]

It seems unlikely, however, that an indwelling foreign body would not in the long run disturb the structure of the tympanic membrane and indeed recent studies indicate that the ventilation tube itself also forms an important aetiological factor in the formation

Table 3. Incidence of tympanosclerotic changes in the tympanic membrane of patients treated with ventilation tubes

Author(s)	N	years after insertion	% Tympanic membrane tympanosclerosis
MacKinnon (1971)	136	n r	33
Mawson & Fagan (1972)	202	n.r.	30
Kilby et al (1972)	47	2	40
Brown et al (1978)	55	5	42
Tos & Poulsen (1979)	527	3 - 8	28
Barfoed & Rosborg (1980)	17	n.r.	61
Møller (1982)	40	2 - 4	40
Lildholt (1983)	132	av 3	53
Tos et al. (1983)	193	1 - 3	47
Slack et al (1984)	124	0 5	31
		1	39
		1.5	47
Kudsen & Lildholdt (1985)	189	3 - 5	52
Skinner et al (1988)	46	15	41
Maw (1991)	158	5	49
Schilder et al (1993)	64	n.r.	48

of tympanosclerosis. Skinner et al.[51] did a controlled trial with patients suffering from bilateral effusion, inserting a tube in only one ear and leaving the other untreated. They found tympanosclerosis in 15.2 % of untreated and 41 % of treated ears after a 15-year-follow-up. Furthermore, Møller[50] found tympanosclerosis in untreated ears in 7.5 % against 40 % in ears 2-4 years after insertion and Lildholdt[33] found 9.8 % in untreated ears vs. 53 % in ears with prior grommet insertion 3 years after treatment Maw[67] also performed extensive studies on OME and its treatment and observed tympanosclerosis in approximately 40-50 % following grommet insertion. He argued that grommet insertion on only one occasion can induce changes which are as severe as those caused by insertion on several occasions. Finally, Söderberg[68] observed that repeated myringotomies and repeated tube insertions in animal experiments caused pronounced formation of tympanosclerotic plaques.

The pathogenesis of tympanosclerosis due to grommet insertion was explained in various ways. Tos et al.[34] observed granulation tissue at the edges of the grommet and considered this to be a foreign body reaction Furthermore, relative immmobility of the drum due to the grommet was postulated as predisposing to tympanosclerosis,

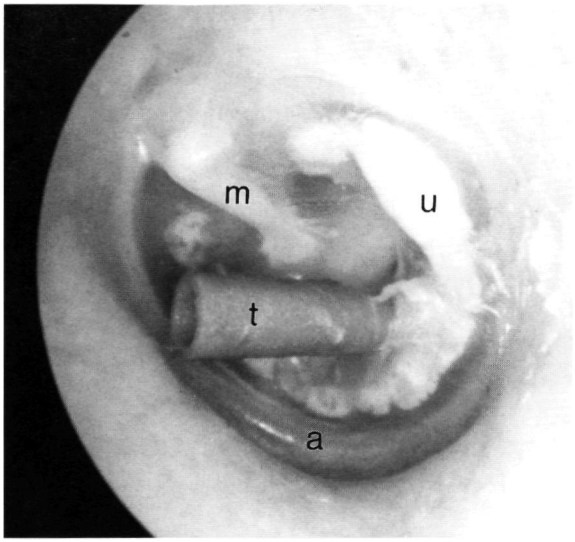

Figure 2
U-shaped tympanosclerotic plaque (u). a: annulus; m: malleus; t: inserted ventilation tube.

which was substantiated by the observation that the horse-shoe shaped tympanosclerosis is seldom situated near the umbo, where the movement of the drum is most pronounced *(Figure 2)*.[34]

The mechanical influence of the ventilation tube was analyzed by Lesser et al.,[31] who produced a mathematical model based on the distribution of tympanosclerosis 15 years after grommet insertions. The plaques were most pronounced in the areas which also exhibited maximal shear stress between the layers of the tympanic membrane. Electronmicroscopical examination showed furthermore that the mass of the grommet could cause sufficient stress to rupture small fibrils within the lamina propria and that the ensuing tympanosclerosis was a repair phenomenon.

Parker et al.[36] showed a significant relationship between the occurrence of tympanosclerosis and the presence of intra-epithelial hemorrhage shortly after insertion. Ears in which hemorrhage did not occur and where the grommet had extruded, did not develop tympanosclerosis. They argued that blood or its degradation products could act in concert with the mass effect of the grommet in initiating tympanosclerotic change.

4. TYMPANOSCLEROSIS AND CHOLESTEATOMA

In surgery for chronic otitis media or its sequelae, tympanosclerosis is occasionally found in combination with cholesteatoma. Because both disorders are common pathological features in chronic middle ear disease, several investigators have studied a possible association, which led to conflicting views.

Zöllner[20] felt that cholesteatoma formation is favoured by the cicatrized distortion of the tympanic cavity in tympanosclerotic ears. According to others, the two conditions could be present at the same time but are not related.[47] Plester[69] found the coincidence of the two disorders rare and more or less accidental. Moreover, hardly any morphological and clinical similarities exist between the two disorders. The most striking dif-

ference is the clinical appearance of the involved ear dry usually, with a large central perforation in cases with tympanosclerosis, whereas in an ear with cholesteatoma the perforation or retraction pocket is usually marginal and often there is a malodorous otorrhoea (Table 4)
Gristwood & Venables[70] analyzed 973 cases of chronic middle ear disease and concluded that on statistical grounds a negative association exists between the two conditions and that the presence of the one reduces the likelihood of the existence of the other

Table 4. Co-existence of tympanic cavity tympanosclerosis and cholesteatoma

Author(s)	N	% Tympanic cavity tympanosclerosis + cholesteatoma
Sheehy & House (1962)	227	28
Beck & Ebert (1964)	71	7
Gundersen (1965)	37	20
Zollner (1969)	62	15
Bonneaud (1971)	70	7
Gibb (1976)	138	14
Kinney (1978)	311	<10
Emmet & Shea (1978)	45	4
Gristwood & Venables (1982)	973	4 5

5. MANAGEMENT OF TYMPANOSCLEROSIS

The treatment of the conductive hearing loss resulting from tympanosclerosis is either surgery or a hearing aid The attitude towards surgery differs among surgeons According to some, tympanosclerosis can even constitute an insurmountable otological problem and a probable contra indication to tympanoplastic surgery [15 71]
In general, surgery for tympanosclerosis must meet the same requirements as exist for any tympanoplastic procedure [64]

(1) Removal of irreversible disease
(2) Reconstruction of the sound transforming mechanism
(3) Prevention of recurrence of the primary disease

Analysis of surgical principles and results in the different reports concerning surgical management of tympnosclerosis shows a variety which reflects the different views that exist among surgeons Reports differ considerably with respect to types of procedure and reconstructive materials employed as well as to the different ways of

reporting hearing results Some authors measure the air-bone gap, others the pure tone averages or average air conduction gain.
We propose a classification for tympanosclerosis in different sites, to facilitate comparison of techniques and results in order to be able to give a more reliable prediction as to the final outcome of a surgical procedure.

Type I Involvement of the tympanic membrane, either intact or perforated. Sometimes this involves the malleus.
Type II. Fixation of the malleus-incus complex in the attic with a mobile stapes
Type III. Fixation of the stapes The malleus-incus complex, if present, is mobile. The stapedial arch may be absent
Type IV. Fixation of the stapes and malleus-incus complex.

5.1 Type I: Tympanic membrane involvement

It is not always necessary to remove tympanosclerotic plaques from the tympanic membrane There are, however, indications for excision: when plaques compromise compliance of the tympanic membrane, when they are so thick that they interfere with middle ear ventilation and when the plaques are likely to impair healing of a graft because of diminished vascularity of the tympanic membrane.[17 47]
Prior to surgical removal, the plaque needs to be separated from the squamous epithelium of the tympanic membrane in order to mobilize it The plaque is then removed either via an existing perforation or via a tympanomeatal flap. Subsequently, myringoplasty can be performed if necessary. In a series of 58 cases carried out as described above, Gibb found a take rate of 96.5%, using the underlay technique.[17]

5.2 Type II: Attic fixation

If there is no antero-superior tympanic membrane plaque or it has been removed and the ossicular chain is still fixed, it is important to determine the site of fixation.
By palpation of the stapes and the long process of the incus, after disarticulation of the incudo-stapedial joint, if necessary, it can be determined whether the ossicles are fixed in the attic, the oval window or both. Total exposure of the epitympanum is sometimes necessary in cases of attic fixation. Some authors have performed a subsequent mobilization of the ossicles, but there is great risk of refixation within a short period of time.[48 71] Others, in order to prevent this, advocate removal of the tympanosclerotic mass at the points where the ossicular chain is fixed, with simultaneous removal of malleus head and incus, after which reconstruction of the sound transforming mechanism is carried out according to the surgeon's preference. Some good results have been claimed with this type of procedure, however, the number of patients reported have been small and the variations employed to reconstruct the ossicular chain preclude adequate comparison of results.[18 72]

5.3 Type III: Fixation of the stapes

Two categories of stapes involvement are distinguished by Smyth:[66,3] one in which only a small amount of tympanosclerosis is present, causing a moderate conductive hearing loss and one in which gross involvement of the stapes is present, enveloping the stapes superstructure and causing a severe conductive hearing loss *(Figure 3)*.

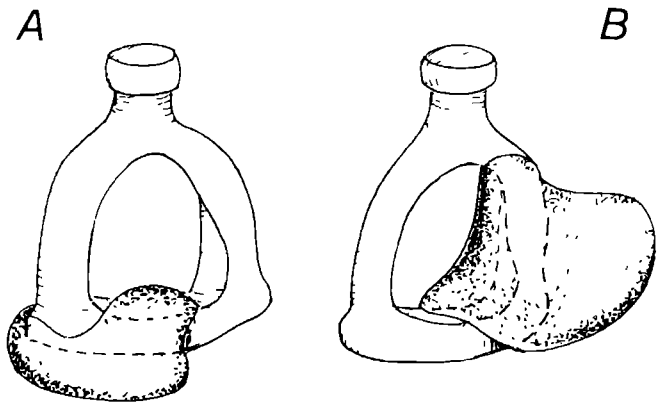

Figure 3 A Tympanosclerosis involving the stapes footplate
B Tympanosclerosis enveloping part of the stapedial superstructure

Tympanosclerosis of the stapedial tendon may be a third category
If surgery on the stapes is decided upon, this should be carried out with an intact eardrum and in a middle-ear free from infection. This usually necessitates staging the procedure,[73] although some claim good results in one-stage procedures.[72] A period of at least 6 months-1 year without signs of infection is considered to be a safe one[74] and broad spectrum antibiotic prophylaxis is considered obligatory.[74]
The procedure starts by assessing the mobility of the stapes for which separation of the incudo-stapedial joint may have to be performed first. Some surgeons advocate a subsequent cautious excision of the tympanosclerotic mass. If the stapes superstructure is affected, a crurectomy is advised with en bloc removal of the process with the arch. This is then followed by mobilization of the stapes and subsequent reconstruction.[8,72]
Reports on hearing results of this technique, however, showed that many patients still had an air-bone gap of more than 10 dB.[16,63] This was thought to be due to either insufficient stapes mobilization or its refixation. Another important disadvantage of a mobilization procedure was the frequent occurrence of post-operative cochlear dysfunction. In 57 patients in which mobilization was carried out, Smyth[63] observed a significant cochlear loss in 53%. This irreversible sensorineural hearing loss was thought to be due either to hydraulic trauma to the cochlea, or a perilymph fistula, both being the result of the unphysiologic movements of the abnormal footplate during the procedure.
Because the results of mobilization with or without crurectomy were unpredictable

and unsatisfactory, it was felt by many that stapedectomy followed by closure of the oval window should be the treatment of choice for tympanosclerosis of the oval window area After stapedectomy, the oval window was sealed off with different materials such as fat, tragal perichondium, fascia or vein As removal of incus and malleus head was often necessary because of co-existing attic fixation, reconstruction of the sound transforming mechanism between the oval window and malleus handle or tympanic membrane was needed A variety of prostheses are used in obtaining this reconstruction such as the incus, cartilage struts total ossicular replacement prostheses (TORP s) and partial ossicular replacement prostheses (PORP's), depending on the quality of the stapedial remnants and the preference of the surgeon [18 72 75]
The overall results in the majority of studies of total stapedectomy procedures were slightly better than the results obtained with mobilization Although short-term results showed that the average air-bone gap in some series was less than 10 dB in the majority of patients reported, Smyth & Gormley[74] observed a marked hearing-deterioration in the long-term only 28% in a series of 46 patients still had an air-bone gap of less than 10 dB, 5-10 years postoperatively Cochlear function in this study was shown to be well-maintained except in frequencies of over 4 kHz at which deterioration was noted
Small fenestra stapedectomy or stapedotomy was performed in only a few patients In the only existing report so far on long-term results of this procedure,[75] improvement of conductive hearing loss was less than observed in stapedectomy, thought to be most likely due to residual fixation of the malleus-incus complex Cochlear function at all frequencies, however, was better maintained in stapedotomy than in total stapedectomy

5.4 Type IV: Fixation of malleus/incus and stapes

Tos et al [72] stated that in cases with limited deposition, excision of tympanosclerosis followed by mobilization of the ossicular chain, which must remain intact, gives satisfactory results, in the long-term In cases with severe disease, an atticotomy is performed, followed by removal of tympanosclerotic deposits malleus head and incus and a subsequent stapedectomy Restoration of the sound transforming mechanism is carried out between oval window and malleus handle or tympanic membrane Studies reporting on long-term results with this procedure, showed a marked air-bone gap reduction at one year, which increased, however, over time [72 75]
In conclusion, all procedures showed that at long-term assessment the air-bone gap deteriorated with time Moreover, in the long-term air conduction levels tended to be worse than the level of socially adequate hearing, generally defined as a mean air-conduction level worse than 30 dB

6. CONCLUSIONS

There is general agreement that tympanosclerosis is an irreversible non-specific end-result of chronic inflammatory processes and traumatic events to the tympanic mem-

brane and middle ear
In most cases of tympanosclerosis in the middle ear, the surgeon will not encounter active chronic ear disease Therefore, surgery is primarily aimed at improving the patient's hearing Considering the above-mentioned studies, it is the view of the authors that conclusions can be drawn only with regard to patients with type I, III and IV tympanosclerosis Reports on patients in which only the attic was involved were too scanty and numbers of patients too small to justify any conclusion or possible recommendation as to the procedure to be performed Hearing improvement in type I patients was generally satisfactory and long-lasting In the remaining types III and IV, reports on results in achieving hearing improvement, clearly indicate a necessity for distinction into short-term and long-term prospects
At short-term, moderate success was obtained in a substantial number of patients, not only in terms of closure of the air-bone gap but also in improvement of the air-conduction level This was true for mobilization procedures as well as for stapedectomies In the long-term, however, these results show a dramatic deterioration with time Some considered refixation of the remaining ossicles to be the cause, but it was also noted that deterioration is a normal feature in any ossiculoplasty procedure in an ear affected by chronic otitis processes and therefore also in cases of tympanosclerosis An alarming number of ears suffering from post-operative sensorineural hearing loss was found in most studies This was shown to be most marked after mobilization procedures and least after a stapedotomy

In considering ossiculoplasty in a tympanosclerotic ear, it should be borne in mind that as in any operation aimed solely at hearing improvement, the patient's hearing disability is also determined by the hearing of the non-operated ear Also, it should be emphasized that air-bone gap closure measures the efficacy of the surgeon's technique but not necessarily the patients' benefit from the operation One can only reasonably expect a significant benefit to be obtained if the operated ear reaches an air-conduction level of 30 dB for the speech frequencies or is within 15 dB of the other ear,[76] taken into consideration that results of surgery for tympanosclerosis generally are worse than those to be expected in other ossiculoplasties, including otosclerosis, the potential benefit for the patient is questionable in most instances When one also considers the risk for iatrogenic sensorineural hearing loss, the indication for surgical correction must be very guarded, even with initial hearing improvement a hearing aid will often still be required in the long term in many of these patients

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CHAPTER II

AN EXPERIMENTAL MODEL FOR TYMPANOSCLEROSIS

Part of this paper has been published in

E W J Wielinga, W Kuijpers, E L G M Tonnaer and P H K Jap

Acta Otolaryngol 1988, 105 537 542

ABSTRACT

The effects of sterile middle ear effusions on the structure of the tympanic membrane have been studied in an animal model The effusions were induced by eustachian tube obstruction in germfree rats It appeared that tympanosclerotic lesions especially marked in the mucosa and the circular and radial fibrous layers were evoked with high reproducibility during the course of this sterile otitis media Occasionally tympanosclerosis was observed in the middle ear mucosa after prolonged survival The histopathological features of this induced lesion were similar to those reported in human specimens It was concluded that this process is most probably caused by a mechanical injury or deterioration of the blood supply related to the underpressure in the middle ear cavity

INTRODUCTION

Tympanosclerosis is a disease of the lamina propria of the middle ear mucosa It affects mainly the tympanic membrane and involvement of the middle ear mucosa is less common It is a frequent sequela of chronic otitis media and is characterised by dense layers of collagenous fibrous tissue in the lamina propria often showing hyalinisation This tissue is commonly poor in cells and shows patchy or diffuse calcification [1] Incidentally areas of bone and cartilage have been observed [2] Despite many light and electronmicroscopical investigations [2-4] including animal studies [5,6] which have contributed considerably to our insight into the character of this condition etiology and pathogenesis are still obscure Degenerative processes in the connective tissue, thought to be triggered by chronic inflammatory processes are generally assumed as important etiological factors Tos and Poulsen[7] suggested that tympanosclerosis is the final process of any infection or inflammation of the middle ear Trauma has been suggested to be another factor contributing to the development of tympanosclerosis This assumption is based on the high incidence of tympanosclerosis after the insertion of ventilation tubes in cases of chronic otitis media [8-11] A peculiar theory has been proposed by Schiff et al [5] where they suggested autoimmunity as an underlying mechanism This hypothesis was based on an experimental study in guinea pigs which were sensitised with an antiserum raised against connective tissue of the tympanic membrane In these animals tympanosclerosis like lesions developed when the tympanic membrane was traumatized

In this study an animal model is presented where tympanosclerosis could be evoked during the course of a sterile serous otitis media induced by obstruction of the eustachian tube

MATERIAL AND METHODS

For this study 60 germ free Wistar rats (b w about 150 g) were used The animals were anesthetised with Hypnorm (0 05 ml/ 100g b w i m) and diazepam (0 05 ml/100g b w i p) The eustachian tube of the left ear was reached by a ventral approach medially to the posterior belly of the digastric muscle under sterile conditions

and obstructed in its extratympanic course as described by van der Beek & Kuijpers[12] The right ear served as a control

To determine the changes in the tympanic membrane and the middle ear the animals were examined under the otomicroscope at weekly intervals during the first two months and thereafter at monthly and bimonthly intervals The animals were killed with a lethal dose of nembutal at intervals varying from one week up to 2 years after obstruction The temporal bone was dissected from the skull fixed in phosphate buffered (0 1 M, pH 7 4) glutaraldehyde (2 5 %) and further processed for lightmi croscopy (LM) and transmission electronmicroscopy (TEM)

For LM the specimens were decalcified in EDTA (10%, pH 7 4) and following dehy dration embedded in glycol methacrylate Sections (2μm) were stained with toluidin blue periodic acid Schiff (PAS) or alcian blue After the temporal bone was fixed and decalcified in a solution containing EDTA (10%) and glutaraldehyde (1 5%) the tympanic membrane was dissected from it for TEM The specimens were postfixed in phosphate buffered (0 1 M pH 7 4) osmium tetroxide (1%) dehydrated and embedded in Epon Ultrathin sections were contrasted with a saturated solution of uranyl acetate and lead citrate and studied with a Philips EM 300 electronmicroscope

For the determination of calcium undecalcified GMA sections were stained by von Kossa s method and undecalcified ultra thin sections were studied with a Philips EM 400 connected to a Tracor Northern (TN) 2000 X-ray microanalyser

To examine the middle ear content for the presence of microorganisms part of the bony wall of the middle ear was removed With a needle a small amount of the mid dle ear content was taken and cultured on blood agar plates, Brewer thioglycollate medium (Difco) and heart infusion broth (Difco)

RESULTS

Normal tympanic membrane

The structure of the tympanic membrane of the rat does not fundamentally differ from that of the human membrane although all tissue layers are much thinner and the size of the pars flaccida is fairly large in comparison with pars tensa Three different layers can be distinguished an outer epidermal layer a middle lamina propria and an inner epithelial layer *(Figure 1A)* The epidermal layer consists of 2-3 layers of kera tinizing epithelium The epithelial lining on the middle ear side is composed of a thin pseudostratified modified respiratory epithelium The lamina propria of the pars tensa is composed of two layers of densely packed collagenous fibres separated from the outer and inner epithelial lining by a very thin layer of loose connective tissue containing small blood vessels and nerve fibres The dense fibrous layer consists of an outer layer of radial fibres and an inner layer of circular fibres *(Figure 1A)* These layers and the subepithelial connective tissue layers are well-developed in the perip heral part of the pars tensa and in the central part near the handle of the malleus In the thinner part of the pars tensa the loose connective tissue and the inner circular layer are very poorly developed and hardly traceable The lamina propria of the pars flaccida which gradually passes into the subepithelial connective tissue of the meatal

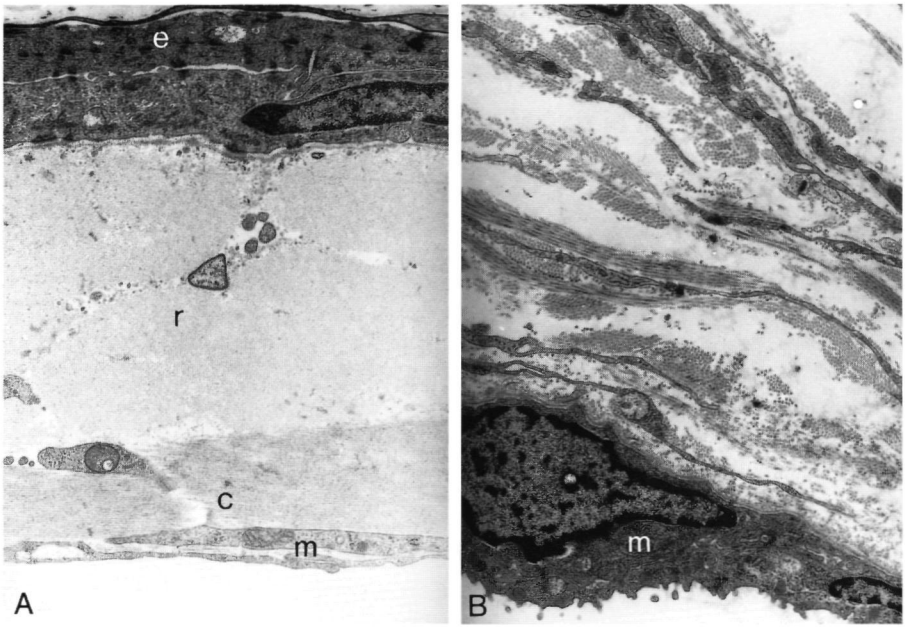


Figure 1. Electronmicrographs of pars tensa (A) and pars flaccida (B) of rat tympanic membrane. The pars tensa is characterised by the presence of a radial (r) and a circular (c) layer of dense fibrous tissue. The pars flaccida is composed of very loose connective tissue with scattered bundles of collagenous fibres. e: epidermis; m: middle ear epithelium. Magnif. A x 4000; B x 11.000.

skin is formed of loose connective tissue with mainly collagenous *(Figure 1B)* and scattered elastic fibres. It contains nerve fibres, many blood-vessels and scattered mast cells.

Tympanic membrane and serous otitis media

From the group of 60 animals operated upon, 20 were excluded from this study because of failed occlusion or the development of an infectious middle ear disease as assessed by otoscopy. The remaining 40 animals developed a serous otitis media, which could be followed from 1 week up to two years after the obstruction of the eustachian tube. Culturing of the middle ear content of 5 randomly selected animals did not show any bacteriological growth.

Otoscopy revealed that a clear yellow serum-like fluid accumulated in the middle ear within one week after eustachian tube obstruction. This was associated with an inward retraction of the tympanic membrane, which was most marked in the pars flaccida *(Figure 2)*. This condition persisted throughout the observation period. In all animals which survived for more than 3 weeks, small white spots could be identified in a horseshoe shaped zone in the central part of the pars tensa. These spots increased in number and size and extended with decreasing density towards both the annulus and malleus handle. In animals which survived for more than six months, large plaques appeared to be formed and the diseased tissue often occupied the major part of the pars tensa *(Figure 3)*.

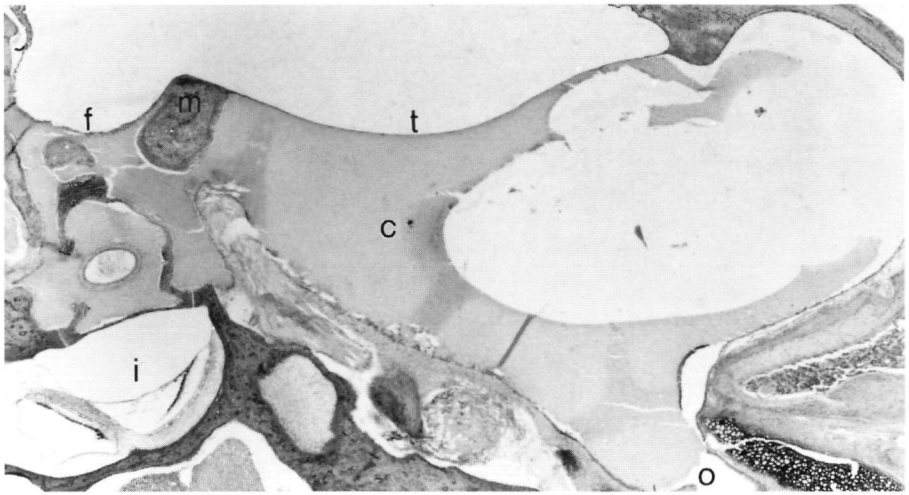


Figure 2.
Lightmicrograph of rat ear 3 weeks after eustachian tube obstruction. The middle ear cavity (c) is filled with an a-cellular serous fluid. The tympanic membrane is retracted, especially the flaccid part (f).
i: inner ear; m: malleus; o: orifice of eustachian tube; t: pars tensa. PAS staining; magnif. x 20.

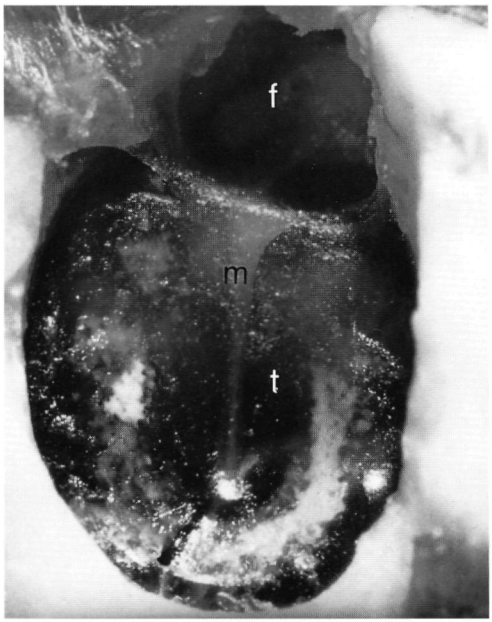


Figure 3.
Otoscopical view of tympanic membrane, 1 year after the induction of a serous middle ear effusion. Note the horseshoe-shaped tympanosclerotic plaque in the pars tensa (t).
m: handle of the malleus; f: pars flaccida.

LM sections showed that the middle ear cavity was completely filled with a fluid free of cells *(Figure 2)* except for some erythrocytes. During prolonged survival up to two years, an increasing number of phagocytic cells and cholesterol clefts became visible. The nature of the fluid was initially thin and serous, but became gradually transformed into a viscid substance. Simultaneously, staining with PAS increased.
With TEM, structural changes were observed after only 1 week. The internal epithelial lining revealed hypertrophy and vacuolisation of the cells. The lamina propria was thickened and showed edema. There was an increased number of fibroblasts with an abundant rough endoplasmatic reticulum and lysosomes *(Figures 4 A,B)*. In addition,

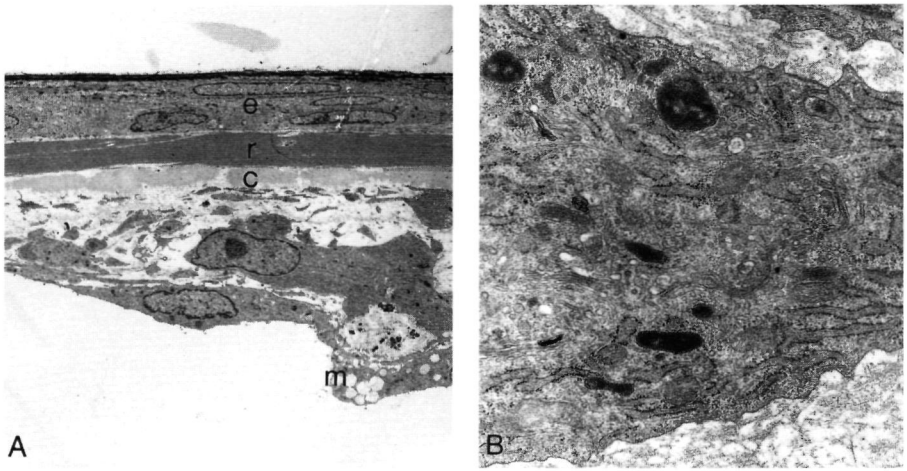


Figure 4. Electronmicrographs of tympanic membrane, one week after eustachian tube obstruction. A shows thickened edematous mucosa and vacuolated middle ear epithelium (m). B shows fibroblast from this area with abundant RER and lysosomes. e: epidermis; r: radial fibres; c: circular fibres. Magnif. A x 1500; B x 20.000.

slight fibril disarrangement of the inner circular layer was observed.
Distinct lightmicroscopical changes first became apparent 2-3 weeks after eustachian tube obstruction. At some sites the mucosa of the pars tensa showed irregularly formed protrusions *(Figure 5)*. These structures, which revealed a PAS-positive ground

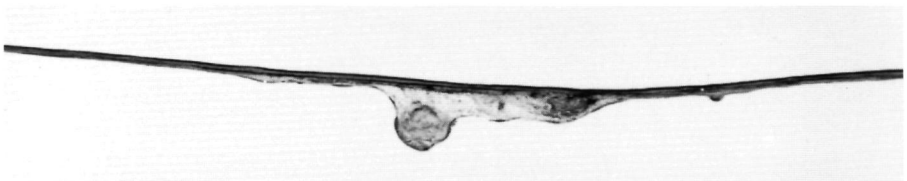


Figure 5. Lightmicrograph of pars tensa showing mucosal protrusions on the side of the middle ear, 3 weeks after eustachian tube obstruction. Toluidin blue staining; magnif. x 80.

substance, contained a few scattered fibroblasts and fibres and often showed calcareous deposits. In the 2-3 week specimens TEM revealed gaps in the inner circular layer, and disintegration of collagen as was concluded from the presence of amorphous electron-dense material and fragmented fibrils between packed microfibrillar structures *(Figure 6 A,B,C)*. In these areas macrophages and newly formed abnormal collagen fibres were present *(Figure 6D)*. Nucleation centres and calcospherules were evident in the thickened mucosa *(Figure 6A,C)*. The calcospherules were either round or fuzzy surfaced or showed a clear lamina limitans, while also fine mineral deposits were observed. The calcospherules were found scattered in the area between the dense fibrous layer and the middle ear epithelium, but they were especially numerous in the circular layer and in the inner side of the radial fibres *(Figure 6A)*. X-ray microanalysis of the calcospherules revealed the presence of high concentrations of calcium and phosphate *(Figure 7)*.

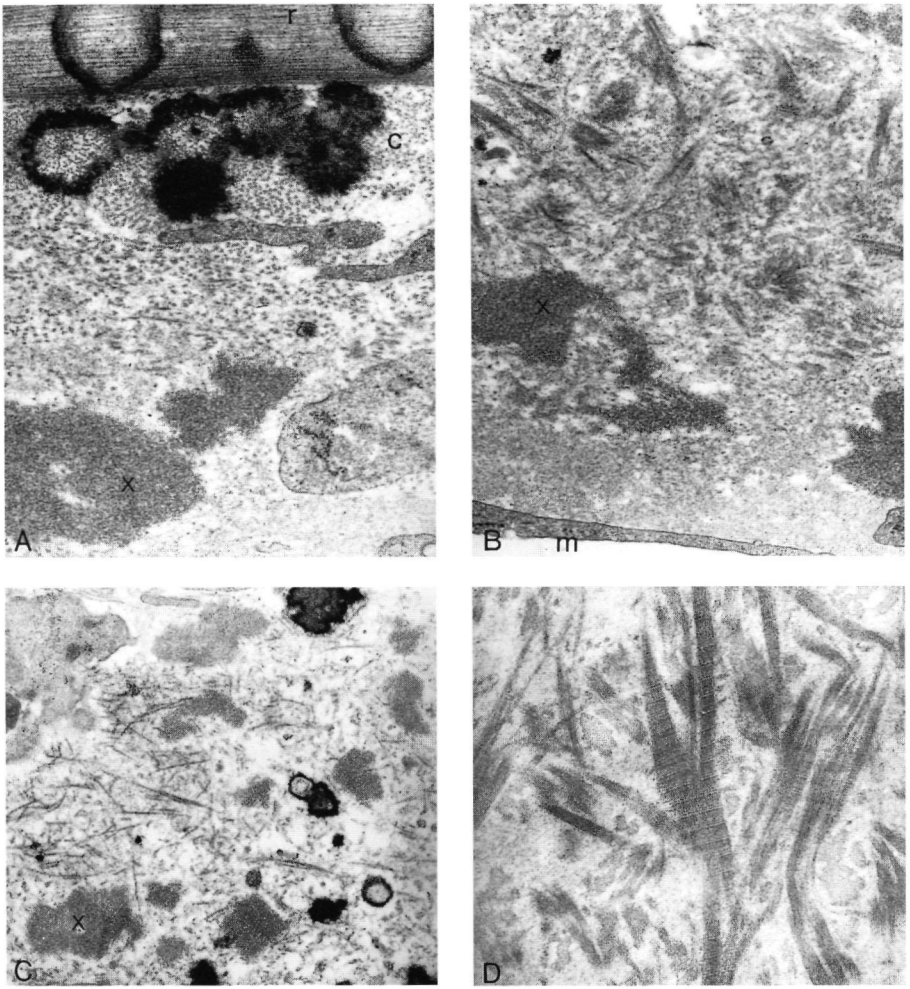


Figure 6. Electronmicrographs of mucosa of pars tensa, 3 weeks after eustachian tube obstruction. The lamina propria is thickened and contains degenerated fibrils (x) (A-C), newly formed abnormal collagen fibrils (B-D) and nucleation centres and calcareous deposits of varying size (A,C). Note calcification in circular (c) and radial (r) fibrous layers (A). m: middle ear epithelium. Magnif. A,B x 20.000; C x 12.000; D x 50.000.

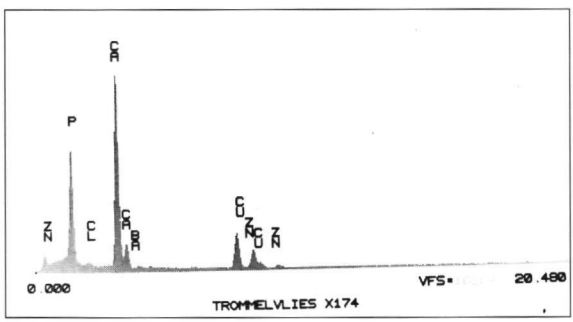


Figure 7.
X-ray mapping of calcareous deposits demonstrating high concentrations of calcium (Ca) and phosphate (P)

In the 2 month specimens the pars tensa was largely thickened due mainly to fibrosis of the lamina propria and to a lesser extent of the subepidermal connective tissue. The fibrous tissue contained a varying number of fibroblasts, but avascular hyalinised areas devoid of cells were also present. Ultramicroscopically a fine fibrillar ground substance and scattered abnormal collagen fibres were observed in these areas. They often showed extensive calcification *(Figures 8 A,B, 9)*. The inner circular and the outer radial fibrous layer demonstrated marked calcification, gaps and fractures *(Figure 8 A,B)*. Degeneration of fibres and formation of abnormal fibres continued. Calcospherules had fused to large complexes in the inner lamina propria and on the inner side of the radial fibrous layer. The inner circular layer was difficult to trace. In this area large calcified plaques were present. In decalcified sections, the calcified areas showed distinct staining with PAS and AB, indicating the presence of proteoglycans.

During prolonged survival, thickening of the lamina propria further increased, due mainly to the formation of calcified hyalinised tissue. EM showed large calcified plaques with medial extensions at the site of the dense fibrous layers (*Figure 10*). Occasionally, mixoid and chondroid areas showing a varying degree of calcification were observed *(Figure 11 A-C)*.

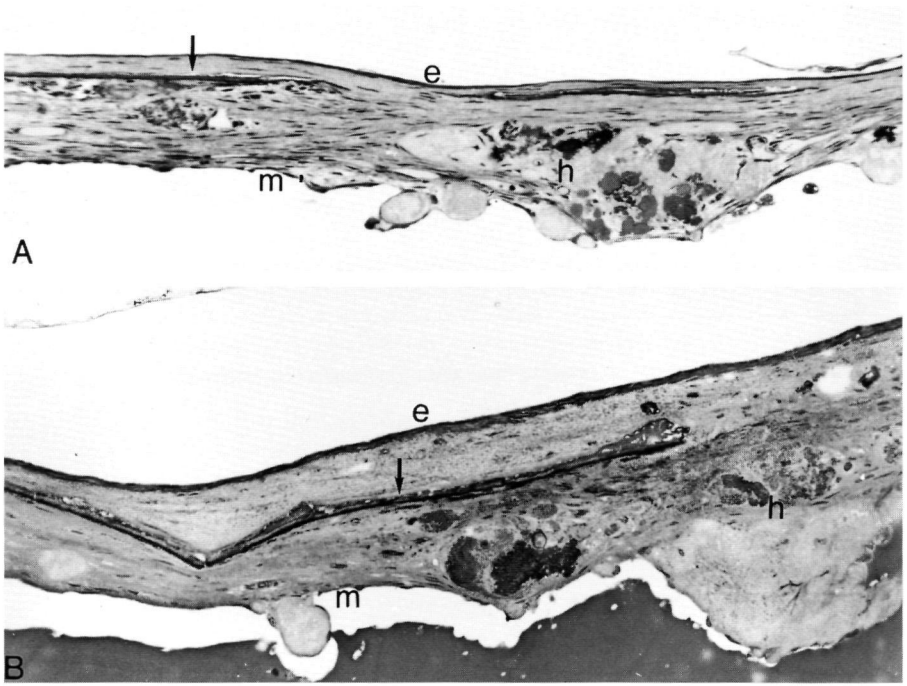


Figure 8. Lightmicrographs of two different pars tensa specimens, 8 weeks after eustachian tube obstruction. The membrane is largely thickened and composed of connective tissue with a locally varying number of fibroblasts and hyalinised areas (h), which show calcification. The calcified dense fibrous layer (arrows) shows gaps and is fractured (B). e: epidermis; m: middle ear epithelium. Toluidin blue staining; magnif. x 200.

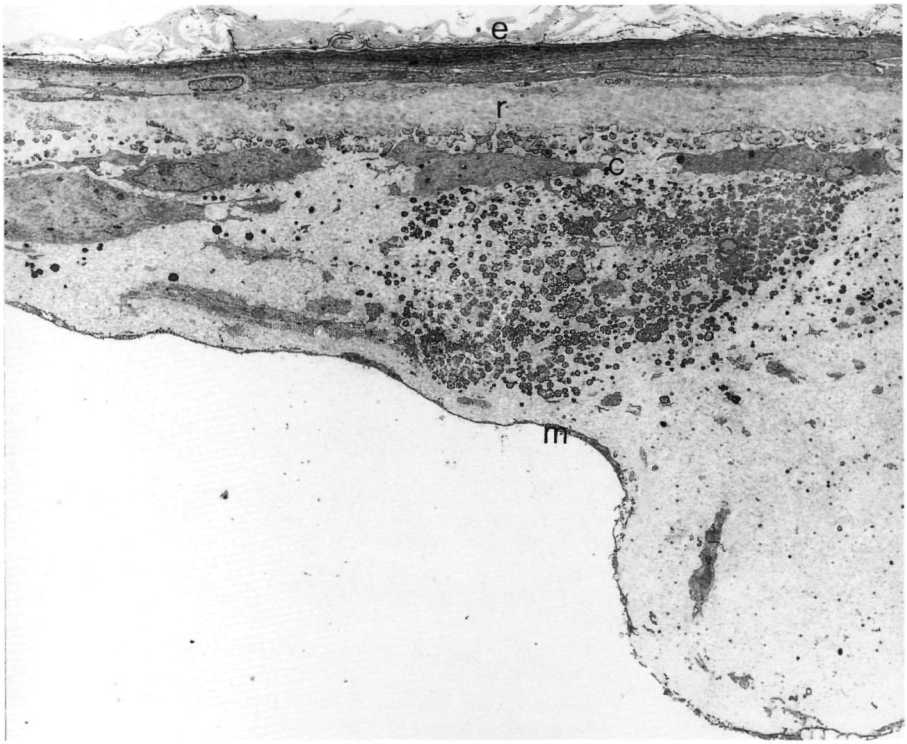


Figure 9. Electronmicrograph of pars tensa, 8 weeks after eustachian tube obstruction. The irregularly thickened lamina propria consists of amorphous material with some fibroblasts and numerous calcareous deposits. The inner circular layer (c) is largely degenerated and shows calcareous deposits. The outer radial layer (r) reveals starting calcification. e: epidermis; m: middle ear epithelium. Magnif. x 1500.

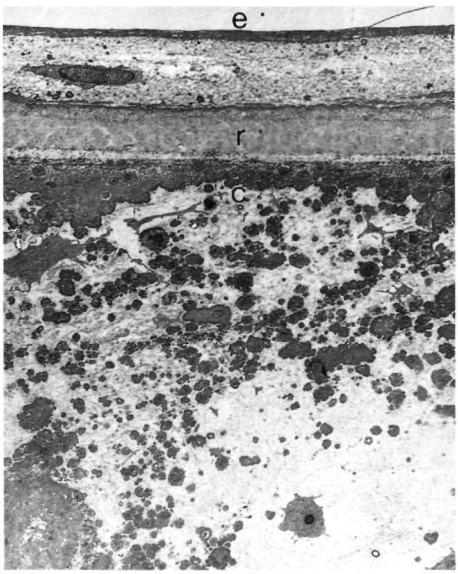
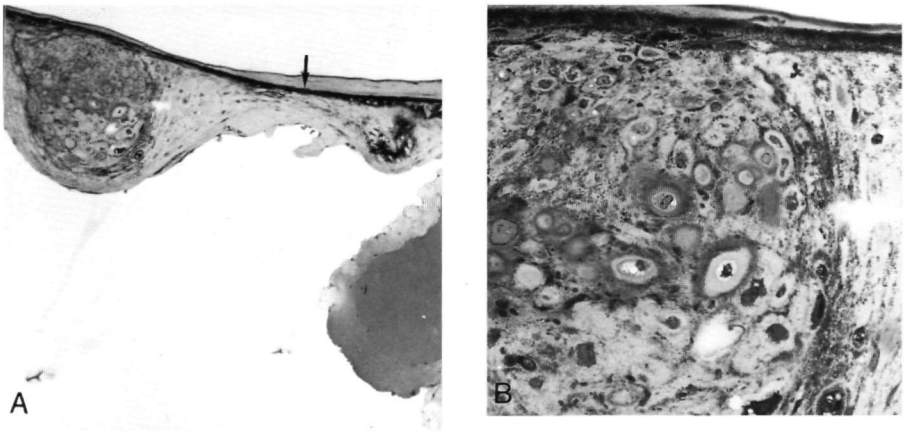


Figure 10.
Electronmicrograph of pars tensa, 4 months after eustachian tube obstruction, showing increased thickness of the subepidermal connective tissue. Note extensive calcification of the circular fibrous layer (c) and lamina propria.
e: epidermis;
r: radial fibrous layer. Magnif. x 1800.



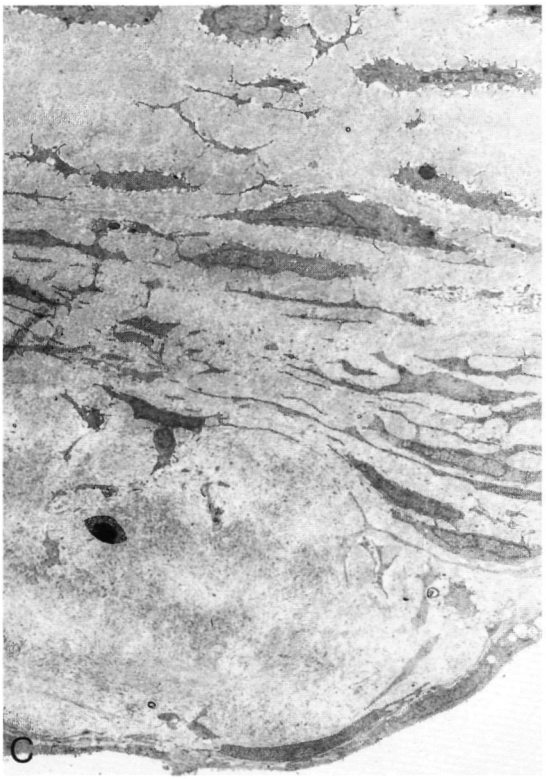


Figure 11.
Lightmicrographs (A: survey; B: higher magnification) of chondroid tissue adhered to calcified dense fibrous layer and electronmicrograph (C) of mixoid tissue in thickened part of the mucosa, 4 months after eustachian tube obstruction. Note scattered calcification in chondroid tissue. A: Toluidin blue staining; Magnif. x 100; B: Toluidin blue staining;
magnif. x 350; C: magnif. x 1500.

The lamina propria of animals which survived for 1-2 years contained connective tissue with a largely varying number of fibrocytes and areas of hyalinised material. Most of the specimens revealed comprehensive calcification of the dense fibrous tissue and the hyalinised tissue in the medial part, but also the subepidermal area was often involved *(Figure 12)* .
This course of events was observed in all ears studied. The nature of the lesion varied

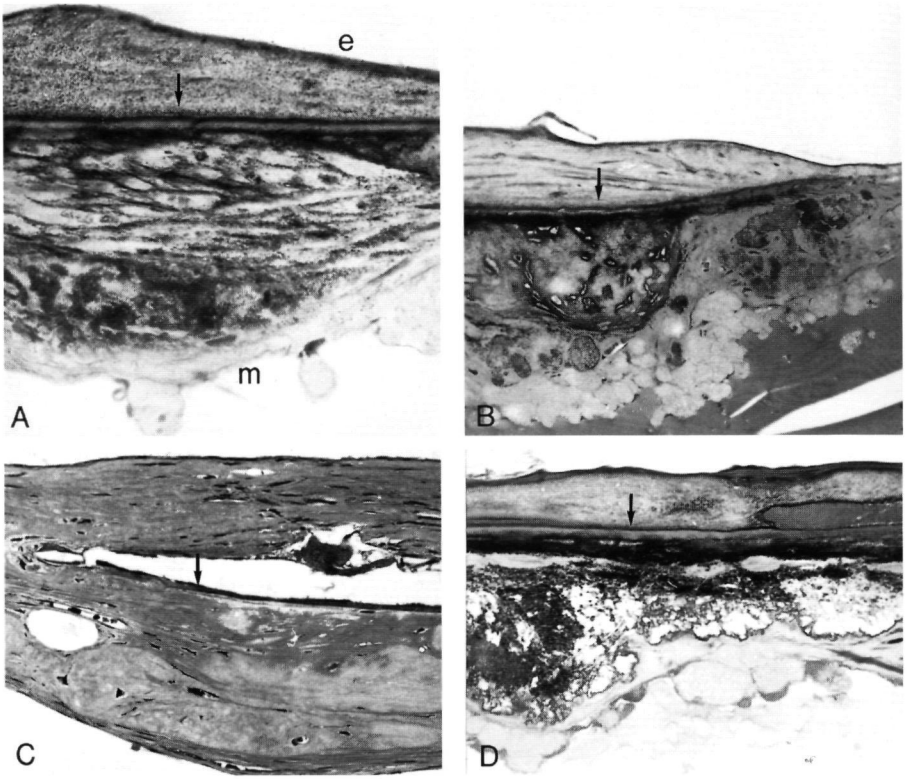


Figure 12. Lightmicrographs of tympanic membrane, one (A, B) and two years (C, D) after eustachian tube obstruction showing comprehensive calcification of dense fibrous layers (arrows) and of hyalinised lamina propria as well as subepidermal tissue in A and D. C shows areas of cellular connective tissue and hyalinised areas; calcification is limited to dense fibrous tissue. e: epidermis; m: middle ear epithelium. Toluidin blue staining; magnif. A,B,D x 350; C x 200.

at different sites of the pars tensa and between different specimens. In all specimens studied, the annular region and the area adjacent to the handle of the malleus remained unaffected. In none of the specimens studied, inflammatory cells were observed.Apart from these observations small perforations of the tympanic membrane were observed in three specimens which survived for 2, 4 and 6 months, respectively. These perforations showed fracturing of the calcified fibrous layer and an inflammatory reaction associated with firoblast activity, as shown in *Figure 13*. In four specimens which survived for more than 1 year, the epidermal layer showed local discontinuities, leaving the bare surface of the hyalinised lamina propria. Throughout the observation period the pars flaccida became gradually thinner, but it retained its original features *(Figure 14)*.

In the mucosa, lining the bony wall of the middle ear, tympanosclerotic changes were found in only 3 animals which survived for 2 years. The lesions were characterized by calcification of the lamina propria and the presence of large calcified polypoid formations lined with epithelial cells. *(Figure 15)*.

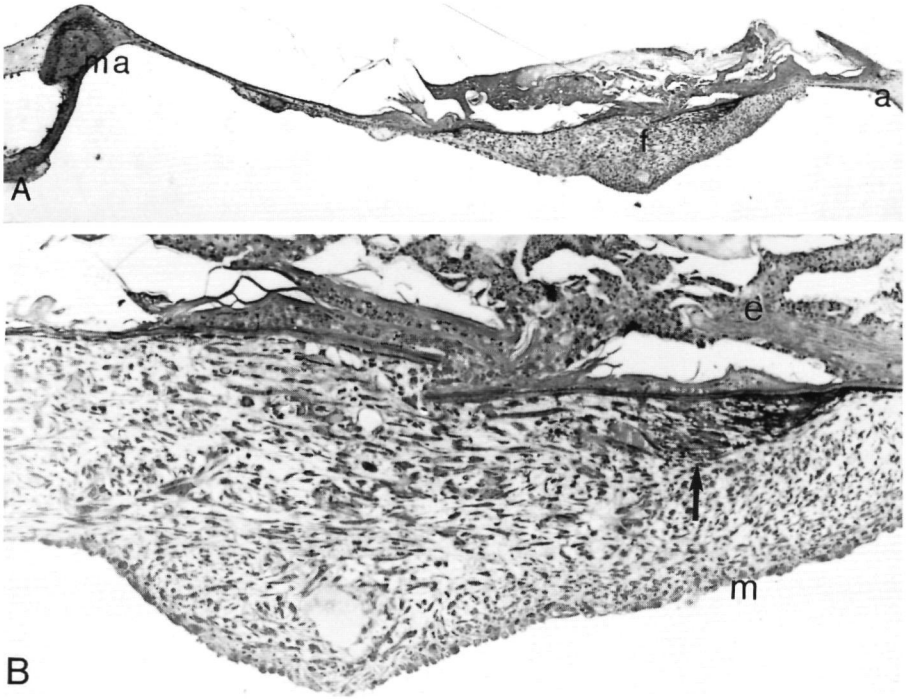


Figure 13. Lightmicrograph of pars tensa (survey A; higher magnification B), 8 weeks after eustachian tube obstruction with fracture (f) of calcified dense fibrous layer and micro-perforation associated with excessive desquamation and high fibroblast activity. Note calcification in this area (arrow). a: annulus; e: epidermis; m: middle ear epithelium; ma: malleus. Toluidin blue staining; magnif. A x 40; B x 160.

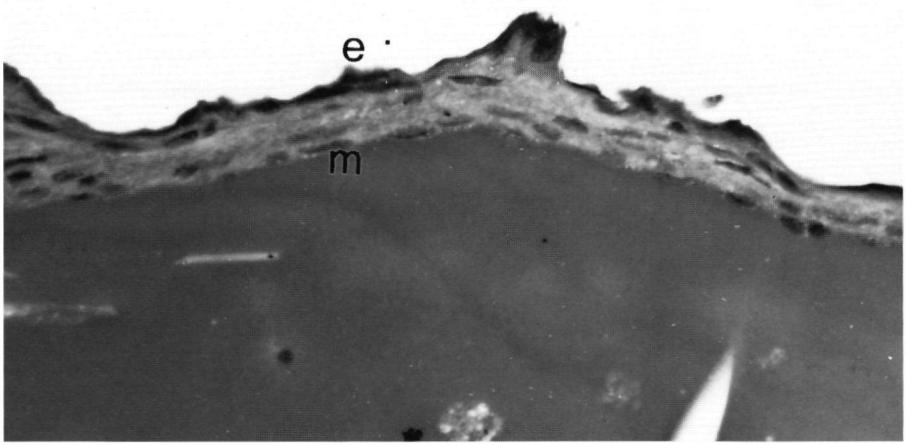


Figure 14. Lightmicrograph of pars flaccida, 8 months after eustachian tube obstruction. The thickness of the membrane is decreased, but the lamina propria has retained its original structure. e: epidermis; m: middle ear epithelium. Toluidin blue staining; magnif. x 400.

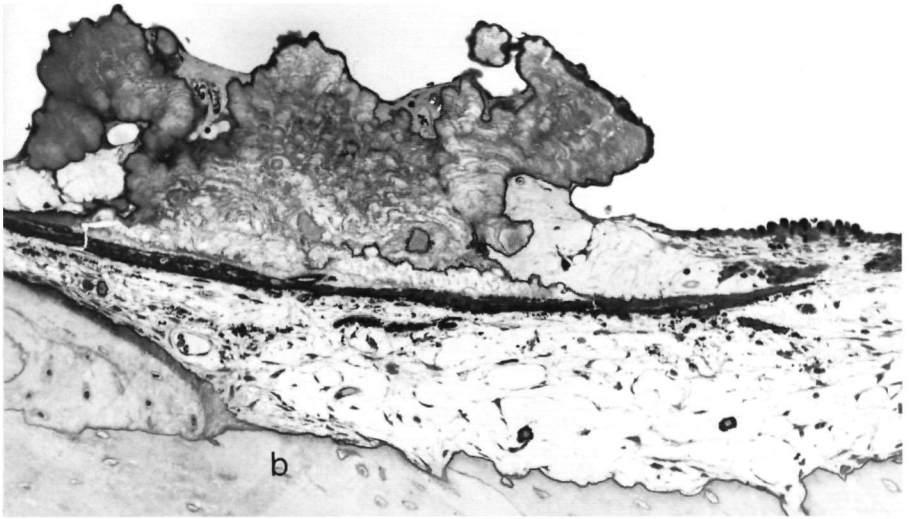


Figure 15. Lightmicrograph of mucosa of the bony wall (b) of the middle ear, showing irregularly lined calcified protrusion, 2 years after eustachian tube obstruction. Toluidin blue staining; magnif. x 200.

DISCUSSION

The present study demonstrates that eustachian tube obstruction in germ-free rats results in the development of tympanosclerotic lesions. The tympanic membrane lesions were confined to the pars tensa and the pars flaccida remained unaffected; the middle ear mucosa only occasionally developed sclerotic lesions after prolonged survival. This animal model allows us to study the development of tympanosclerotic lesions from the very beginning. The process is characterised by degeneration of fibrils, formation of abnormal fibrils and calcification. It finally results in a largely thickened pars tensa composed of fibrous tissue showing a varying degree of hyalinisation and calcification.

Although infection and inflammation are excluded as causal factors, it remains difficult to decide on the trigger of this process. Obstruction of the eustachian tube is known to cause reduced pressure in the middle ear because of gas absorption.[13] This results in an inward retraction of the tympanic membrane and transudation of serum from the mucosal vessels. Both the accumulated fluid in the middle ear cavity and the continuing retraction of the pars tensa can be considered as possible triggers. This fluid has close contact with the inner layer of the tympanic membrane and it might be assumed that some components of this fluid such as hydrolytic enzymes may exert an injurious effect on the tympanic membrane. However, this assumption seems not very likely because the accumulated fluid has intimate contact with the whole inner surface of the tympanic membrane, while the lesion starts and remains confined to a horseshoe-shaped area of the pars tensa midway between annulus and the handle of the malleus. The margins of the pars tensa are not affected. Alternatively, the particular site of this lesion suggests that mechanical deformation caused by retraction of

the tympanic membrane is a more likely explanation for its development Due to the inward retraction of the tympanic membrane the pars tensa becomes exposed to an unphysiological tension This tension will be largest in the central area of the pars tensa midway between annulus and malleus handle and this coincides with the site of injury

This assumption also allows for an explanation for the absence of lesions in the pars flaccida This structure can easily collapse because the middle layer is composed of loosely textured fibrous tissue and lacks the tensed fibres of the pars tensa Therefore it can readily adapt to the applied tension by retraction without damage to the fibres In the pars tensa, this tension will lead to disruption and damage of the tensed radial and circular fibres The tympanic membrane responds to this injury by increased fibroblast activity and the formation of new collagen fibres However, because the retraction of the pars tensa persists there will be a continuing process of injury and attempts to repair the damage This eventually results in a profound change of the structure of the fibrous part of the tympanic membrane, while the epidermis can also be affected

The origin of these lesions may be the direct result of mechanical injury but, in addition deterioration of the blood supply due to the retraction of the tympanic membrane may be responsible for, or add to the development of, this lesion The thinnest part of the pars tensa, the site of the lesion, has only a very poor vascular supply in contrast to the areas adjacent to the annulus and the handle of the malleus[14] and severe mechanical deformation may be assumed to interfere with the blood supply, resulting in a persistent hypoxia

In order to explain the persistent retraction of the tympanic membrane we must assume that after the fluid has accumulated there remains a pressure gradient between middle ear and the ambient air although this gradient will be smaller than that immediately after eustachian tube obstruction but before the inflow of the fluid Support for this assumption can be derived from the observations made by Buckingham & Ferrer[15] and Sade et al [16] They observed such a gradient in patients with secretory otitis

The calcification process is reminiscent of dystrophic calcifying lesions which can occur in damaged or devitalised soft tissues in other sites of the body [17 18]

The site of the lesions and the histopathological features of this experimental tympanosclerosis are very similar to those described in humans by various authors as a sequela of chronic otitis media [2 4 19] The same applies for the chemical compostion of the calcified deposits [20] Although tympanosclerosis is generally assumed to be a sequela of chronic inflammatory and infective processes in the middle ear, the present experimental study convincingly demonstrates that these processes are not mandatory for the development of these lesions Longstanding negative middle ear pressure associated with retraction of the tympanic membrane must be considered as an important factor in the development of tympanosclerotic lesions in chronic otitis media

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CHAPTER III

STRUCTURAL CHANGES OF THE TYMPANIC MEMBRANE IN THE PRESENCE OF STERILE AND INFECTED MIDDLE EAR EFFUSIONS

E W J. Wielinga, W. Kuijpers, T.A. Peters and E L.G.M. Tonnaer

Submitted

ABSTRACT

This study deals with an animal model in which the influence of chronic otitis media on the structure of the lamina propria of the tympanic membrane was studied, in relation to the development of tympanosclerosis. Middle ear effusions were induced by obstruction of the eustachian tube of specific pathogen free rats and rats with upper respiratory tract infection. The effects of serous effusions, primary and secondary infected effusions as well as re-aeration of the middle ear on the tympanic membrane were studied by means of light- and electronmicroscopy. It was demonstrated that sterile effusions always resulted in tympanosclerotic lesions. These lesions did not develop in the presence of primary infected effusions. They had a varying destructive effect on the lamina propria, followed by fibrosis of the tympanic membrane. Generally, supervening infection did not markedly affect pre-existing lesions. Moreover, calcification disappeared when re-aeration of the middle ear occurred, but the abnormal collagen depositions persisted. Mechanical injury and compromised vascularisation of the tympanic membrane owing to inward retraction, rather than infection, are assumed to be important etiological factors in the development of tympanosclerosis in chronic otitis media

INTRODUCTION

Otitis media and trauma are the main causes of tympanic membrane injury. Tympanic membrane perforations either of traumatic origin or resulting from acute otitis media often heal spontaneously usually leaving no trace or only a minor scar. However, chronic otitis media can lead to persistent pathological changes like atrophy, tympanosclerosis, adhesive otitis and perforations.[1] These changes can seriously interfere with sound transmission and the function of the middle ear mucosa. Chronic otitis media is a well recognized clinical entity. It can present in different forms, on account of the large variation in the nature of the middle ear effusions, which can vary from a clear effusion without distinct signs of infection to a cloudy or purulent fluid.[1] Numerous studies have dealt with the clinico-pathological changes of the tympanic membrane, but little is known about the pathogenesis of these lesions and only a few histopathological reports are available. In biopsies taken from the tympanic membrane during tympanostomy in cases of chronic otitis media, Sano et al.[2] established that the submucosal layer was mostly affected. It showed edema, fibrosis and decreased thickness of the circular and radial fibre layers. Sadé[1] observed the absence of these fibres in the tympanic membranes of patients with atelectasis. Similar observations have been made in temporal bone studies, although the complete absence of fibrous elements is still a matter of discussion.[3-5]

Animal studies have also been focussed on the effects of infection on the structure of the tympanic membrane. However, in these studies, middle ear infections were induced by the injection of suspensions of various exogeneous bacterial strains into the middle ear cavity.[6-10] The middle ear infections induced in this way were usually of a low-grade nature and recovered spontaneously within a short period. The structure of the lamina propria of the tympanic membrane was reported to be little affected. It

can be questionned as to whether these studies can be considered as representative of chronic otitis media

Previous studies in our laboratory showed that eustachian tube obstruction which is an important etiological factor in chronic otitis media [1] is an appropriate model for the induction of middle ear effusions in rats [8,11] Therefore this approach which is a better approximation of the pathophysiology was used to study the effect of chronic otitis media on the tympanic membrane

MATERIAL AND METHODS

For this study, 126 rats were used, divided into two groups One group (N=101) consisted of specific pathogen free animals (SPF) but they were housed in a conventional environment without special protective measures The second group (N=25) consisted of rats contaminated with Mycoplasma pneumoniae in their upper airways and showing clinical symptoms of upper respiratory tract infection (URTI) The experiments were conducted according to institutional guidelines on animal experimentation At the time of operation all animals had healthy middle ears as assessed by otoscopy The animals were anesthetised with Hypnorm (0 05 mL/100 g, i m) and diazepam (0 05 mL/100 g i p) The eustachian tube was reached by a ventral approach as described previously Obstruction of the eustachian tube of the left ear was performed in the extratympanic course by electrocautery using a fine needle coagulator [11]

Postoperatively the condition of the middle ear was monitored otomicroscopically at regular time intervals This inspection was performed daily during the first postoperative week thereafter at weekly intervals and after two months at monthly intervals After varying survival times the animals were killed by an intracardiac injection of sodium pentobarbital (Nembutal) For lightmicroscopy (LM) a tissue block containing the middle ear and the medial part of the external ear canal was dissected and fixed in phosphate buffered (0 1 M, pH 7 4) glutaraldehyde (2%) Subsequently the specimens were decalcified in EDTA (10%, pH 7 4), dehydrated and embedded either in paraffin wax or in glycol methacrylate (GMA) Paraffin sections (7µm) and GMA sections (2 µm) were stained with toluidin blue or periodic acid Schiff (PAS)/alcian blue For the demonstration of calcium according to von Kossa's method, the decalcification step was omitted

For transmission electronmicroscopy (TEM), the tympanic membrane and the bony annulus were dissected and fixed in phosphate buffered (0 1 M pH 7 4) glutaraldehyde (2%) Decalcification was performed in a solution containing EDTA (10%) and glutaraldehyde (1 5 % pH 7 4) The specimens were postfixed in phosphate-buffered (0 1 M, pH 7 4) osmium tetroxide (1%), dehydrated and embedded in Epon Ultrathin sections were contrasted with a saturated solution of uranyl acetate and lead citrate and studied with a Philips EM 300 electronmicroscope

For bacteriological studies part of the bony wall of the middle ear was chipped off and with a needle a small amount of the middle ear content was taken and cultured on blood agar plates, Brewer thioglycollate medium (Difco) and Heart infusion broth (Difco) Swabs taken from the nasopharynx were cultured on the same media

RESULTS

Based on otoscopical observations of the tympanic membrane different categories could be distinguished The observations made on the SPF animals are indicated in Table I From 101 SPF ears operated on 10 remained aerated because of failed occlusion and were excluded from this study Forty-one showed the presence of a clear yellow serum like fluid throughout the observation period These ears were denoted as serous ears In nine animals the middle ear became re aerated after a serous period

Table I **Follow-up of SPF animals after eustachian tube obstruction**

Total number	Serous	Serous re-aer	Serous-inf	Prim inf	Failed
101	41	9	23	18	10

which varied from 2 weeks up to 12 weeks In 23 ears middle ear infection started after an initial serous period which varied from 2 weeks up to 16 weeks while in 18 ears an infective middle ear disease developed immediately after eustachian tube obstruction

From the group of animals with URTI all ears (n= 25) developed an infective middle ear disease immediately after obstruction

Serous ears

The animals were sacrificed after survival times varying from 1 week up to 20 months after obstruction

The reaction pattern in the middle ear and tympanic membrane was very similar to the observations made on germ free animals in a previous study[12] (Chapter II) In short inward retraction of the tympanic membrane and an accumulation of a serum-like fluid in the middle ear cavity was observed within one week after eustachian tube obstruction Subsequently in the horse-shoe shaped central part of the pars tensa the development of a tympanosclerotic lesion was observed The pars flaccida remained unaffected but in the middle ear mucosa these lesions were occasionally observed after prolonged survival The lesion was characterised by degeneration of collagen fibres increased fibroblast activity, formation of abnormal collagen fibrils and calcification of both preexistent and newly formed fibrous tissue After survival periods between 4 and 20 months the structure of the pars tensa was profoundly changed The membrane was largely thickened and composed of fibrous tissue with cellularity ranging from almost nil to moderate with circumscript calcification Bacterial culturing of the middle ear content and histology failed to show any sign of infection

Serous-infected ears

The duration of the serous and infective periods of these ears are indicated in Table II Culturing of the middle ear content of 6 randomly selected ears showed the presence

Table II. **Ears infected after a serous period of more than 2 weeks (w)**

number	Serous period	Infectious period
7	2w	2w
4	5w	2-3w
12	8-16w	10-48w

of unclassified gram-negative rods in all ears. From 3 ears, Proteus mirabilis and Staphylococcus albus were also cultured in varying numbers. These micro-organisms were also cultured from the nasopharynx. Distinct re-canalisation of the eustachian tube could be established in LM sections of the majority of specimens which had an infective period of more than 10 weeks.

Ears with a serous period of 2 weeks followed by an infective period of 2 weeks showed a fluid-filled middle ear cavity with a varying number of inflammatory cells and macrophages. In the tympanic membrane, polymorphonuclear leucocytes and lymphocytes were mainly present in the subepidermal and submucosal connective tissue. These areas showed an increased number of fibroblasts and capillaries. The circular and radial fibrous layers often showed less severe infiltration by inflammatory cells.

The tympanic membrane of specimens with a serous period of 5 weeks followed by an infective period of 2-3 weeks showed a moderate to severe infiltration of inflammatory cells. The epidermis revealed hyperplasia. The subepithelial tissue layers on both sides showed increased thickness due to fibrosis and contained capillaries. The fibrous reaction was most intense on the medial side. On this side the mucosa showed local thickenings containing hyalinised areas, which incidentally contained calcareous deposits. Calcification was also present in part of the dense fibrous layer *(Figure 1)*. One specimen showed a very active infective process. The epidermis was hyperplas-

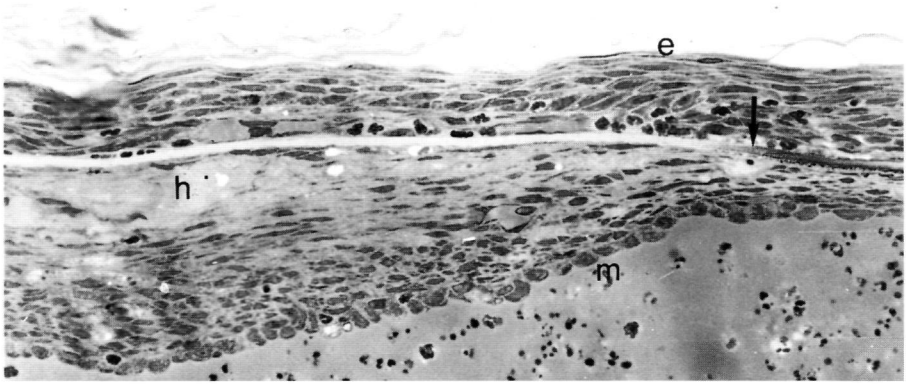

Figure 1. Lightmicrograph of tympanic membrane after a serous period of 5 weeks followed by infection for 2 weeks. The membrane contains scattered inflammatory cells and reveals extensive fibrosis and hyalinisation (h) in the medial part. The epidermis (e) shows hyperplasia. The dense fibrous layer (arrow) shows local calcification. m: middle ear epithelium. Toluidin blue staining; magnif. x 380

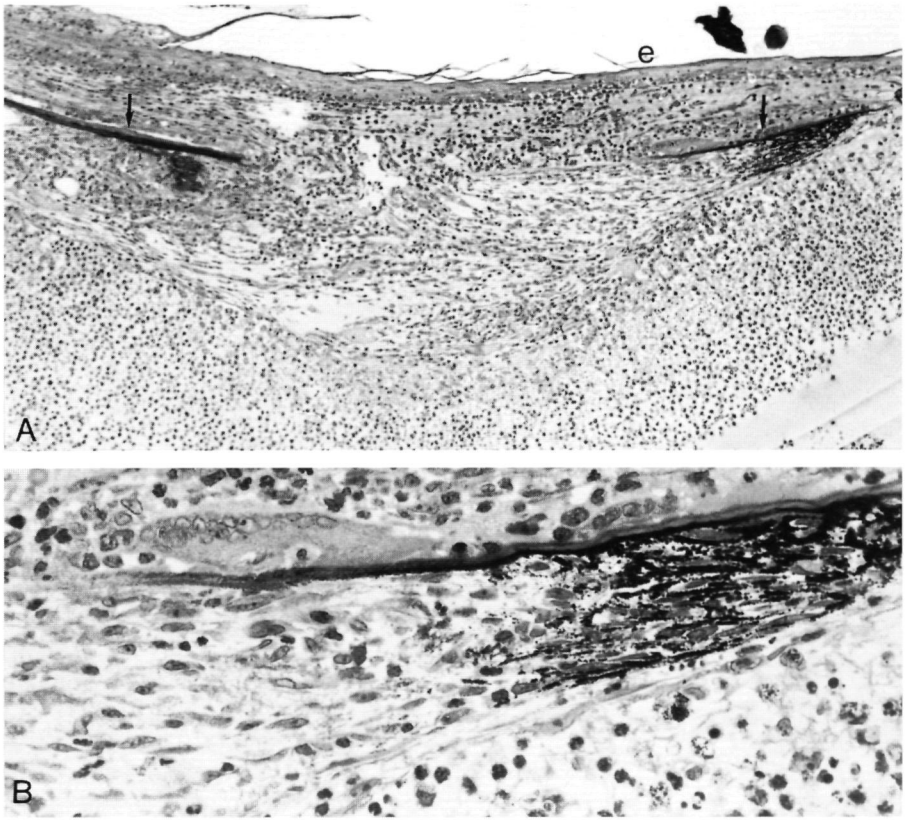

Figure 2. Lightmicrographs (survey A, detail B) of tympanic membrane after a serous period of 5 weeks followed by infection for 3 weeks. The membrane is highly inflamed and the calcified dense fibrous layer (arrow) shows a large gap. Higher magnification in B shows giant cells apposed to the calcified fibrous layer. e: epidermis. Toluidin blue staining; magnif. A x 90, B x 400.

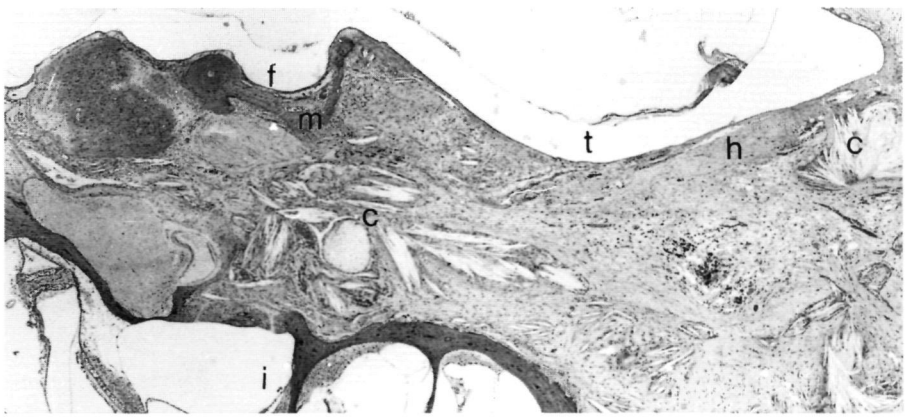

Figure 3. Lightmicrograph of middle ear after a serous period of 8 weeks followed by infective period of 40 weeks. The middle ear is completely filled with fibrous tissue containing cholesterol clefts (c). Both pars tensa (t) and pars flaccida (f) are retracted. h: hyalinised area; i: inner ear; m: malleus. Toluidin blue staining; magnif. x 25.Figure 3

tic and infiltrated by inflammatory cells. The remaining part of the membrane showed edema, many fibroblasts, capillaries and numerous inflammatory cells. The middle ear epithelium was interrupted and part of the calcified dense fibrous layer had disappeared. Locally, giant cells were apposed to this calcified tissue *(Figure 2)*.
From 12 ears with a serous period for 8 - 16 weeks followed by an infective period up to 48 weeks, 8 contained pus, while 4 showed showed a varying degree of fibrosis of the middle ear cleft, an example of which is shown in *Figure 3*. These phenomena appeared unrelated to the survival period. The tympanic membrane was largely thickened in all ears, due to fibrosis. In the purulent cases, the membrane was nearly

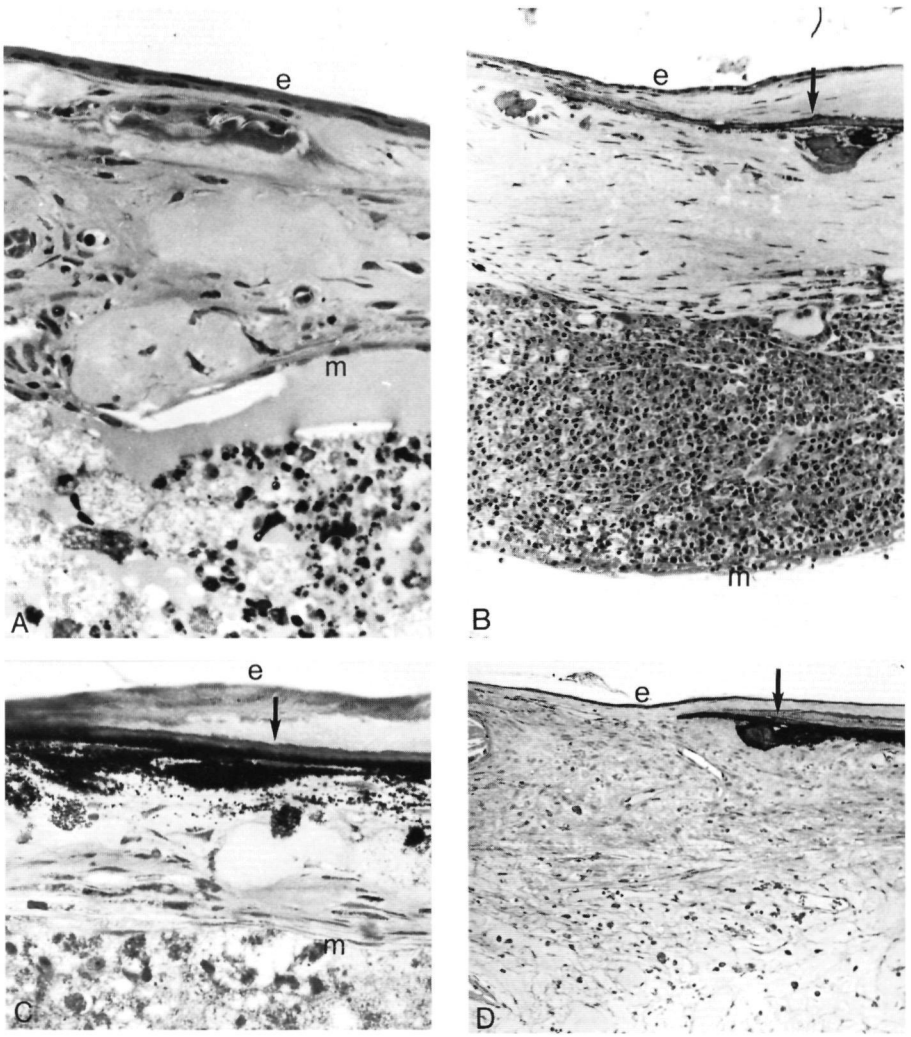

Figure 4. Lightmicrographs of tympanic membranes after varying serous periods (A, C: 16 weeks; B,D: 10 weeks) followed by varying infectious periods (A: 20 weeks; B: 30 weeks, C: 40 weeks; D: 48 weeks). The membranes are composed of connective tissue with varying cellularity and hyalinised areas. Note extensive calcification of the dense fibrous layers in B-D (arrows). e: epidermis; m: middle ear epithelium. Toluidin blue staining; magnif. A,C x 380; B,D x 190.

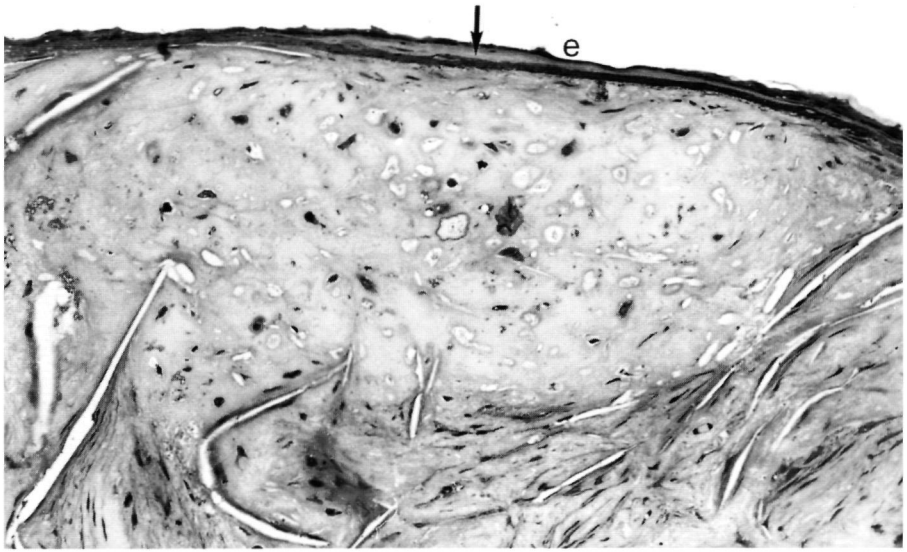

Figure 5. Lightmicrograph of tympanic membrane with chondroid area apposed to calcified dense fibrous layer (arrow) after a serous period of 16 weeks and an infective period of 48 weeks. e: epidermis. Toluidin blue staining; magnif. x 200.

always lined by middle ear epithelium *(Figure 4 A,B,C)*, but in the fibrotic ears the lamina propria was continuous with the fibrous tissue obliterating the middle ear cleft *(Figures 3, 4 D)*. The lamina propria was composed of fibrous tissue with a largely varying number of fibroblasts, capillaries and scattered inflammatory cells and macrophages. In some of the purulent ears, large numbers were found accumulated in the subepithelial area *(Figure 4 B)*. Hyalinised areas were present in all specimens. Areas with a mixoid or chondroid appearance adherent to the dense fibrous layer were ocassionally observed *(Figure 5)*. These areas and the hyalinised areas were usually devoid of inflammatory cells. Calcification was present in all ears, but the degree largely varied between different specimens. It was mainly present in the dense fibrous layer and some medial extensions. This layer was often displaced and showed gaps *(Figure 4)*. The pars flaccida was apposed to the ossicular chain *(Figure 3)*, but no significant structural changes could be established in this part of the membrane.

Serous-re-aerated ears

The durations of the serous and re-aerated period are indicated in Table III. Re-canalisation of the eustachian tube could be established in LM sections of all specimens. In all re-aerated ears, otoscopy revealed a partial or nearly total return of the tympanic membrane to its original position. From 3 specimens with a serous period of 2 weeks followed by re-aeration for 2 weeks, 2 did not show any abnormality. One showed some small mucosal protrusions with a hyalinised content *(Figure 6A)*. Calcareous deposits were found in one of these outgrowings. The remaining part of the

Table III. **Ears re-aerated after an initial serous period in weeks (w)**

Number	Serous	Aerated
3	2w	2w
2	3-4w	2w
4	9-12w	16-32w

membrane was apparently normal. Similar lightmicroscopical observations were made on the specimens which had a serous period of 3-4 weeks followed by re-aeration for 2 weeks.

Generally, the structure of the four specimens with a serous period of 9-12 weeks, followed by re-aeration for 16 and 32 weeks did not show large individual differences.

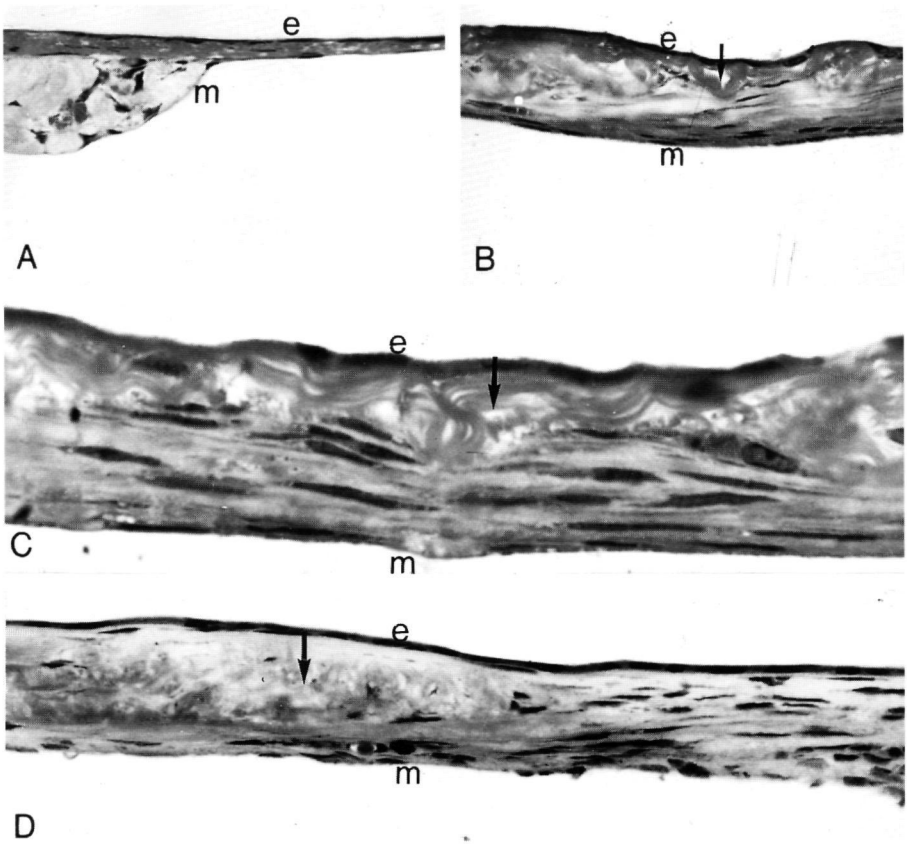

Figure 6. Lightmicrographs of tympanic membrane after varying serous periods followed by varying periods of re-aeration. (A: serous 2 weeks, re-aerated 2 weeks; B: serous 9 weeks, re-aerated 16 weeks; C,D: serous period 12 weeks, re-aerated 32 weeks). A shows mucosal protrusion with hyalinisation. Micrographs in B-D show folded and disintegrating dense fibrous layer (arrows) and connective tissue of varying cellularity and hyalinisation. e: epidermis; m: middle ear epithelium. Toluidin blue staining; magnif. A x 180; B x 350; C x 950; D x 400.

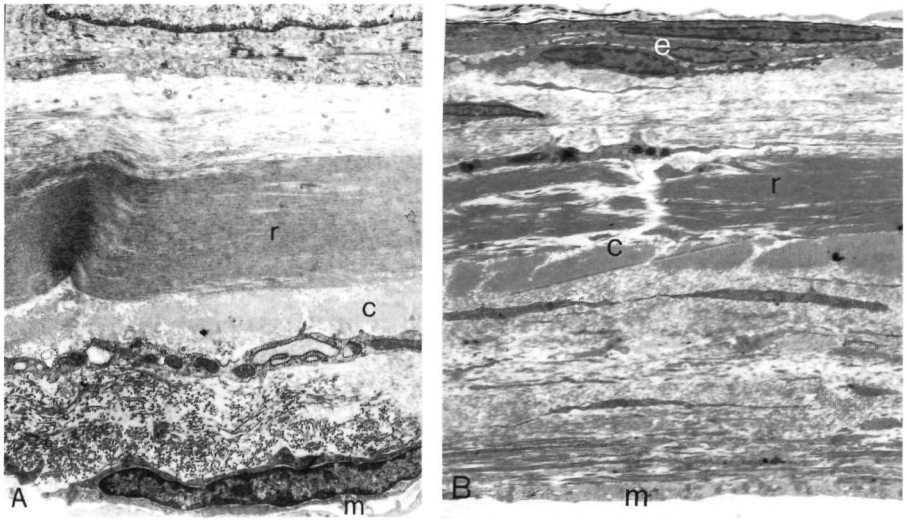

Figure 7. Electronmicrographs of tympanic membranes after varying serous periods followed by re-aeration (A: 12 weeks serous; 32 weeks re-aerated. B: 9 weeks serous, 16 weeks re-aerated). Note folding (A) and distortion of circular (c) and radial (r) fibrous layers (A,B). e: epidermis, m: middle ear epithelium. Magnif. A x 5000; B x 2500.

Parts of these membranes were studied by both LM and TEM. LM showed that the membranes were locally still much thicker than the normal membrane. They were lined by a thin epithelial layer on both sides. The lamina propria was composed of irregularly arranged connective tissue with a few capillaries and hyalinised areas. The number of fibroblasts largely varied at different sites in the membrane. In none of the specimens studied were calcareous deposits present *(Figure 6B-D)*. The original radial and circular fibrous layers showed large gaps and were often folded or severily distorted *(Figure 6B-D)*. Distortion and folding were also observed ultramicroscopically. The subepidermal and subepithelial layers contained irregularly arranged fibres *(Figure 7)*. The circular and radial fibres revealed an abnormal structure.

Primary infected ears

SPF animals and URTI animals, which developed an infected effusion immediately after eustachian tube obstruction were killed after survival times of 1 week, 2 weeks,

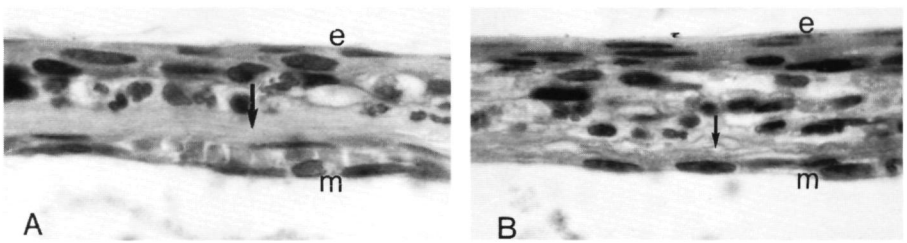

Figure 8. Lightmicrographs of primary infected tympanic membranes of SPF rats, one week after eustachian tube obstruction. Note inflammatory cells dilated capillaries and dense fibrous layer (arrow), apparently intact in A and disintegrating in B. e: epidermis; m: middle ear epithelium. Toluidin blue staining; magnif. A,B x 950.

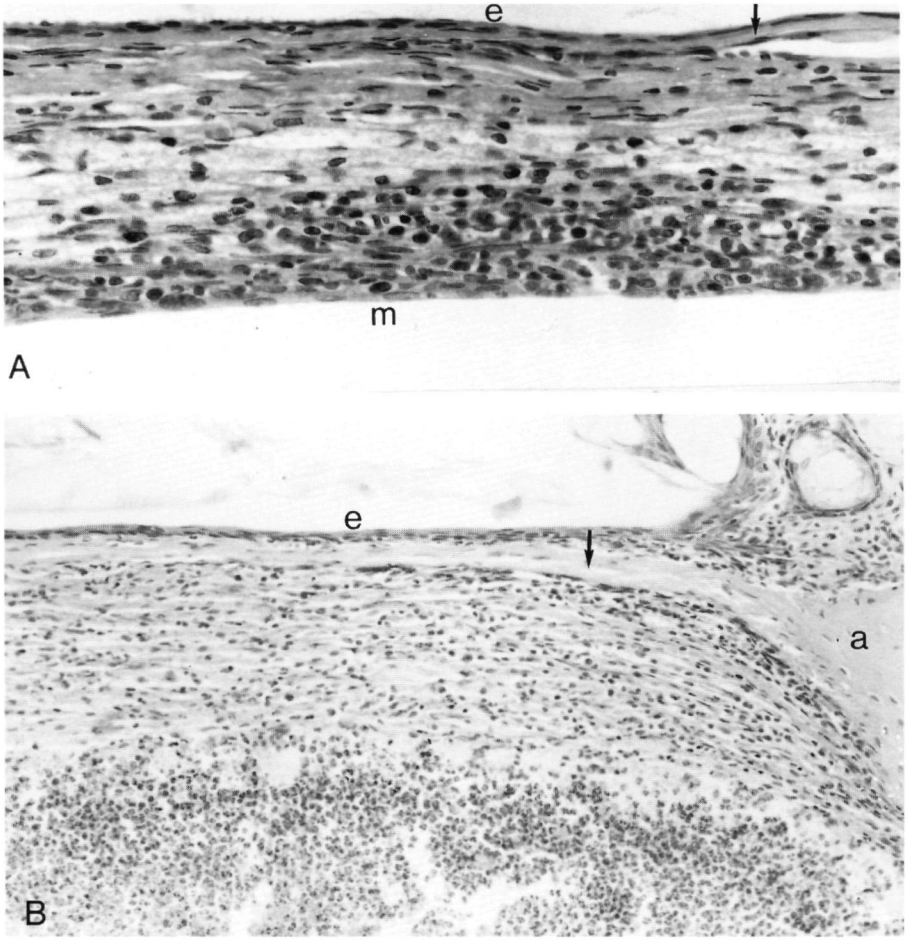

Figure 9. Lightmicrographs of primary infected tympanic membranes of SPF rats, 4 weeks after eustachian tube obstruction. The lamina propria is largely thickened and contains numerous inflammatory cells. The dense fibrous layer (arrows) is unaffected. Note the absence of middle ear epithelium (m) in B. a: annulus; e: epidermis. Toluidin blue staining; magnif. A x 380; B x 100.Figure 9

4 weeks, 16 weeks and 48 weeks. In both groups, otoscopy showed vascular injection, loss of transparency and outward bulging of the tympanic membrane, which was most marked in the pars flaccida during the first days after obstruction. Thereafter, the clinical course differed between both groups. SPF animals showed an opaque tympanic membrane which persisted throughout the observation period. All animals with URTI showed rupture of the tympanic membrane with otorhoea starting between three and ten days. Otorhoea ceased during the second or third postoperative week, usually followed by spontaneous closure of the tympanic membrane. In five ears, no closure took place throughout an observation period of up to 16 weeks.Bacterial culturing of the middle ear content from 5 randomly selected animals from each group showed differences in the bacterial content of both groups. The SPF group revealed generally the same bacteria as found in the serous-infected group. Animals with URTI

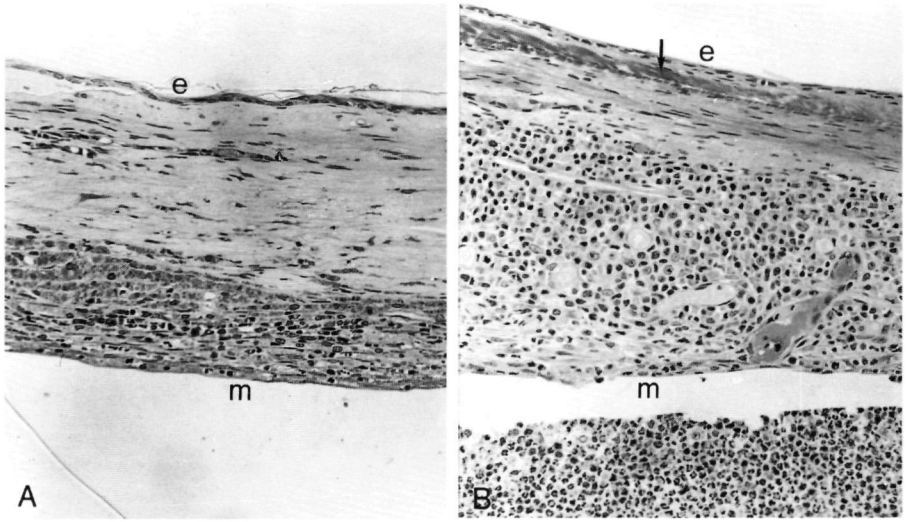

Figure 10. Lightmicrographs of primary infected tympanic membranes of SPF rats, 4 months after eustachian obstruction. The lamina propria is composed of fairly homogeneous connective tissue with scattered capillaries. The original dense fibrous layer can be recognized in B (arrow). Note subepithelial accumulation of inflammatory cells. e: epidermis; m: middle ear epithelium. Toluidin blue staining; magnif. A,B x 200.

also revealed gram negative rods and M. pneumoniae, along with individually varying numbers of an unclassified Streptococcus, an unclassified gram negative rod, Staphylococcus aureus and Staphylococcus albus, Proteus mirabilis, Pasteurella pneumotropica, Streptoccus viridans and occasionally Moraxella sp. and Escherichia coli.Light microscopy from the SPF group showed a middle ear cavity filled with pus, one week after obstruction. The subepidermal layer and the mucosa were infiltrated by inflammatory cells and showed dilated vessels and an increased number of fibroblasts. The dense fibrous layer was affected to a largely varying extent. At some sites this structure was intact, while other sites revealed local distortion or disintegration *(Figure 8A,B)*. During subsequent weeks, the tympanic membrane became largely thickened due to the formation of fibrous tissue, which contained many inflammatory cells *(Figure 9)*. In some cases the middle ear epithelium was absent *(Figure 9B)*. After survival times of more than 2 months, the fibrous tissue obtained a more mature appearance, although inflammatory cells remained present, mainly in the subepithelial layer *(Figure 10)*. In the majority of the ears with an infective period of more than 4 months, the tympanic membrane consisted of a thick fibrous membrane, lined on both sides by a thin epithelium. In two ears, the lamina propria of the tympanic membrane gradually passed into the fibrous tissue obliterating the middle ear. The original dense fibrous layer was present in all ears studied, but often showed distortion or gaps of varying size, especially in the thinnest part of the pars tensa. Throughout the observation period, the pars flaccida was infiltrated by inflammatory cells mainly at the medial side and showed an increased number of capillaries and fibroblasts *(Figure 11)*. In one ear, which survived for 32 weeks, complete healing of the membrane was established. It was composed of homogeneous connective tissue without inflammatory cells *(Figure 12)*

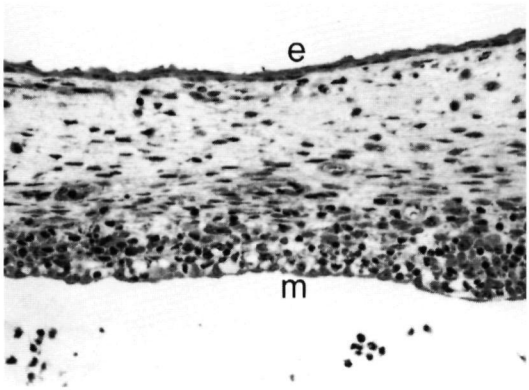

Figure 11.
Lightmicrograph of primary infected pars flaccida of SPF rat, 3 months after eustachian tube obstruction. The membrane is thickened by an increase of connective tissue. Note subepithelial accumulation of inflammatory cells. e: epidermis; m: middle ear epithelium. Toluidin blue staining; magnif. x 380.

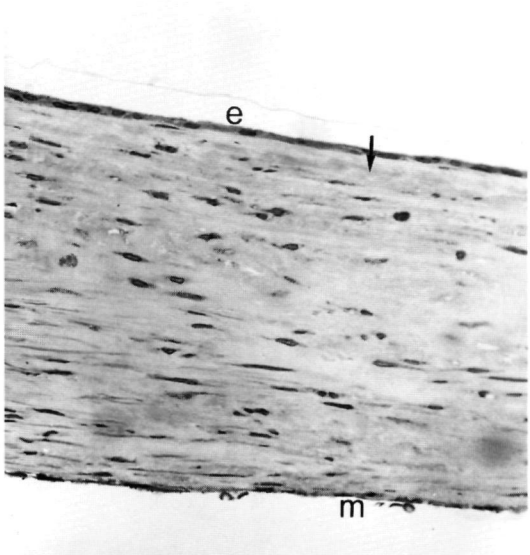

Figure 12.
Lightmicrograph of healed tympanic membrane of primary infected SPF rat, 32 weeks after eustachian tube obstruction. The membrane is composed of homogeneous connective tissue, lined by a thin layer of epidermal (e) and epithelial cells (m). arrow: dense fibrous layer. Toluidin blue staining; magnif. x 380.

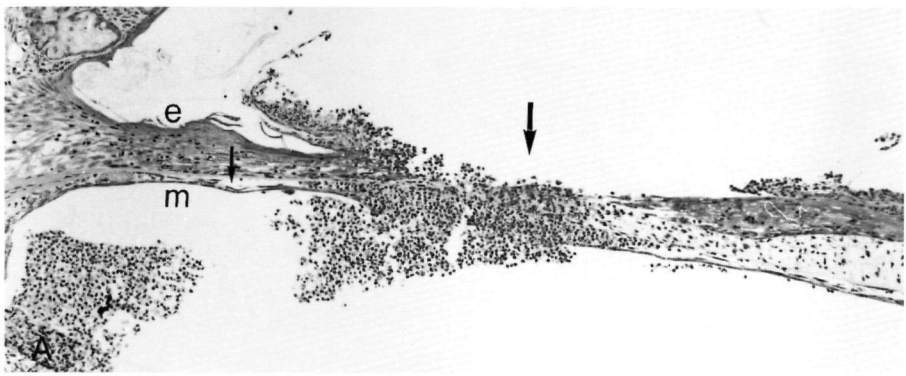

Figure 13. Lightmicrograph of infection induced tympanic membrane perforation (large arrow) of URTI rat, one week after eustachian tube obstruction. e: epidermis; m: middle ear epithelium. Small arrow: dense fibrous layer. Toluidin blue staining; magnif. x 90.

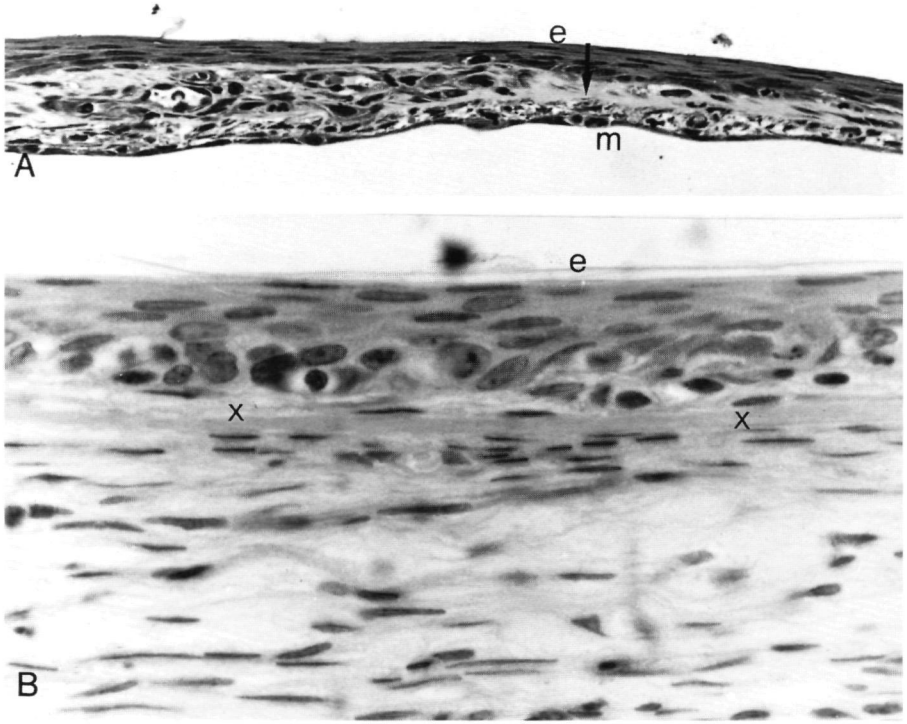

Figure 14. Lightmicrographs of URTI rat, 2 weeks (A) and 8 weeks (B) after closure of infection-induced perforations. A: Lamina propria shows fibroblasts, capillaries, scattered inflammatory cells and remnant of original dense fibrous layer (arrow). B: Lamina propria is composed of fairly homogeneous loosely textured connective tissue. Note subepidermal band of dense fibrous tissue (x). e: epidermis; m: middle ear epithelium. Toluidin blue staining; magnif. A x 380; B x 950.

In the URTI group, the major part of the tympanic membrane was destroyed one week after eustachian tube obstruction *(Figure 13)*. Closure of the perforations was characterised by proliferation of epidermal cells, fibroblasts and capillaries. Two weeks after closure, the lamina propria showed numerous fibroblasts, capillaries and scattered inflammatory cells. The original dense fibrous tissue was still present in the areas close to the handle of the malleus and the annulus *(Figure 14 A)*. In ears where perforations persisted, the edges of the perforation remained thickened and the epidermis had grown around the perforation edge and was present on the medial surface of the tympanic membrane. After prolonged survival, all ears with a closed tympanic membrane studied showed a varying degree of fibrosis of the middle ear cleft, leaving only a minor space filled with cellular debris. The lamina propria of the tympanic membrane was composed of fairly homogeneous connective tissue, containing a varying number of inflammatory cells. Just below the epidermis a band of nearly a-cellular collagenous tissue was usually formed *(Figure 14 B)*. TEM of this area showed the presence of irregularly arranged bundles of densely packed collagenous fibres *(Figure 15)*. In none of the cases complete healing was established. Throughout the observation period, the pars flaccida was infiltrated by inflammatory cells, especially in the medial part, and showed an increased number of fibroblasts and capillaries.

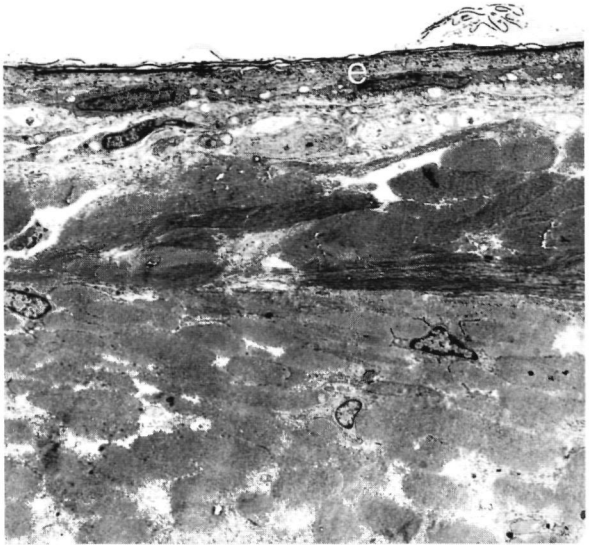

Figure 15.
Electronmicrograph of tympanic membrane of URTI rat, 6 months after closure of an infection-induced tympanic membrane perforation. Note large amount of irregularly arranged densely packed collagenous fibres in the subepidermal area. e: epidermis. Magnif. x 1500.

DISCUSSION

The observations made in the present animal model reveal a striking similarity with the clinical features of chronic otitis media and support the significance of this animal model for studying the effect of chronic otitis media on the structure of the tympanic membrane.

Eustachian tube obstruction results in reduced pressure in the middle ear, inducing transudation of a sterile serum-like fluid, but in the case of supervening infection, which appears to be promoted by URTI, a purulent effusion develops.

The development of primarily infected effusions must be ascribed to the surgically induced disturbance of the clearance function of the eustachian tube, but the question whether this infection is caused by bacteria that pass the injured area from the nasopharynx and ascend into the middle ear cavity or by bacteria already present in the tubotympanum before the surgical intervention cannot be answered.

The induction of both sterile and infected effusions results in profound changes in the fibrous tissue of the pars tensa, but the structural features largely differ. Sterile effusions always result in tympanosclerotic lesions. These lesions are absent in primarily purulent effusions. The nature of the tympanosclerotic lesions, which can be defined as calcifying fibrosis, is identical to that observed in a similar study on germ-free rats[12] (Chapter II). It has been proposed in the latter study that the development of tympanosclerosis is due to injury to the lamina propria and/or vascular disturbance caused by inward retraction of the pars tensa.

The present observations demonstrate that tympanosclerosis can recover when reaeration of the middle ear occurs and the inward retraction of the membrane is relieved. This finding is in line with some clinical observations showing disappearance of tympanosclerosis in a limited group of patients with chronic otitis media after re-

aeration of the middle ear.[13-15] However, the present histological data demonstrate that restoration to the original state of the structure of the pars tensa does not occur. The main feature of the healing process is the disappearance of the calcareous deposits, but hyalinised areas appear to persist. In addition, the circular and radial layers of dense fibrous tissue are still present but they are severily distorted and show severe structural changes. Therefore, they can not fulfill their original role of determining the mechanical strength of the membrane.

According to the assumption that the formation of calcareous deposits can be caused by a disturbed metabolism due to deterioration of the blood supply,[16 17] the disappearance of these deposits can be attributed to improvement of the blood supply. Although it is still unknown whether, and to what extent, the metabolism of the tympanic membrane also depends on the gaseous composition of the middle ear, any such contribution to the recovery of the membrane cannot be excluded.

The severity of the effects of infected effusions on the tympanic membrane appeared highly related to the bacterial strains involved. The main feature of the structural changes of the pars tensa is a partial or nearly total replacement of the lamina propria by a layer of homogeneous connective tissue. This tissue lacks the dense fibrous tissue, arranged in circular and radial layers, but often a new layer of dense fibrous tissue appeared to be formed subepidermally.

Although both sterile and infected effusions result in damage to the dense fibrous layer and additional fibrosis, hyalinisation and calcification do not develop in the presence of infected effusions. The main difference between both processes is, that infected effusions are associated with increased vascularisation, while inward retraction of the tympanic membrane in the absence of infection has been proposed to have an adverse effect on the blood supply of the pars tensa[12] (chapter II). This suggests that deteriorated vascularisation may play a crucial role in the development of tympanosclerotic lesions. Apart from this, interference of the infective process with the substrate, creating the appropriate conditions required for the deposition of calcium phosphate, can be an additional explanation for the lack of calcification.

Generally, supervening infection does not appear to markedly affect longstanding preexisting tympanosclerotic lesions. Partial destruction was only observed in one early lesion, by a severe infective process.

These experimental data are in conflict with the generally accepted theory that tympanosclerosis is the final result of chronic inflammatory or infective processes.[18 19] However, we must consider that tympanosclerosis is a frequent sequela of chronic otitis media in children.[20-24] In this age group, the clinical course is often characterised by a persisting negative pressure in the middle ear with presence of effusion, but without distinct signs of infection. This clinical course supports the present experimental data.

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CHAPTER IV

STRUCTURAL CHANGES OF THE LAMINA PROPRIA AFTER HEALING OF TYMPANIC MEMBRANE PERFORATIONS

E W J Wielinga, W Kuijpers and E L G M Tonnaer

Submitted

ABSTRACT

Traumatic defects of the tympanic membrane usually close without apparent abnormalities in the epithelial lining. Little is known, however, about the structure of the lamina propria after healing of a traumatic perforation of either the pars tensa, or the pars flaccida. The objective of this experimental study was to investigate the healing process of traumatic defects in both pars tensa and pars flaccida with emphasis on the restoration of the lamina propria. It was demonstrated that the lamina propria of the pars tensa showed persistent pathological changes after healing of a traumatic defect, while the pars flaccida healed without structural changes. The lamina propria of the pars tensa did not regain its original typical fibre arrangement. At distance from the defect the devitalised fibrous layer persisted and showed severe calcification. Calcification was also observed in part of the vital fibrous tissue These findings may contribute to a better insight in the development of tympanosclerotic lesions after myringostomy in chronic otitis media

INTRODUCTION

The tympanic membrane has an excellent healing capacity. Perforations, whether of traumatic or infectious origin, show an apt ability to close spontaneously, usually within a short period of time. Moreover, defects caused by trauma usually heal without residual functional consequences. Otoscopical observations on the structural repair of the tympanic membrane after trauma reveal diverging data. According to Kristensen (1992)[1] myringeal repair must generally be characterized as 'restitutio ad integrum'. Other reports described the healed membrane of normal thickness,[2-4] variable thickness,[5] or thin and transparant.[6] It is commonly agreed that pathologic features in the tympanic membrane after healing of a defect stem from structural changes within the lamina propria.

Histological data on the healing process of traumatic perforations of the pars tensa are limited to light microscopical observations in animal studies and mainly focussed on the behaviour of the epidermal cells. As a result of these studies the epithelial lining has been well established as being the first to bridge a defect, showing no abnormalities after completion of healing.[7-10] Remarkably, in these studies only minor attention has been paid to the structural repair of the lamina propria of the pars tensa. The dense fibrous tissue of the circular and radial layer determines largely the mechanical properties of this structure necessary for optimal sound transmission.

In the present experimental study the healing characteristics of traumatic perforations of the pars tensa were investigated, with special emphasis on the repair of the lamina propria. Furthermore, healing of perforations in the pars flaccida, which have been largely disregarded so far, were included. In addition to lightmicroscopy, transmission electronmicroscopy was used for the study of this healing process.

MATERIAL AND METHODS

For this study 50 adult Wistar rats with bilateral healthy middle ears were used. They were operated on according to institutional guidelines on animal experimentation. The animals were anesthetised with hypnorm (0.05 ml/100 g, i.p.) and diazepam (0.05 ml/100g, i.m.). Both ears of all animals were operated on, using the otomicroscope. In 35 ears small central perforations were made in the antero-superior part of the pars tensa, midway between the annulus and the handle of the malleus. In another 35 ears larger marginal perforations were made close to the annulus and the handle of the malleus. In 30 ears small perforations were made in the central part of the pars flaccida. Otoscopical assessment, which was performed daily for the first week and weekly at later stages, showed that no intercurrent infection developed in any ear throughout the observation period. At various time intervals ranging from 7 hours up to 1 year, the animals were sacrificed, the temporal bone removed and fixed in phosphate-buffered glutaraldehyde (2.5 %, pH 7.4), decalcified in EDTA (10 %, pH 7.4) and processed for lightmicroscopy (LM) or transmission electronmicroscopy (TEM). For lightmicroscopy specimens were embedded in glycol methacrylate (GMA). GMA sections (2 µm) were stained with toluidin blue. Von Kossa's method for the demonstration of calcium was applied on sections prepared from undecalcified specimens. Processing for electronmicroscopy was performed by fixation of the dissected tympanic membrane and bony annulus in phosphate-buffered (0.1 M; pH 7.4) glutaraldehyde (2%). Specimens were decalcified in a solution containing EDTA (10%) and glutaraldehyde (1.5%; pH 7.4), postfixed in phosphate-buffered (0.1 M; pH 7.4) osmium-tetroxide (1%), dehydrated and embedded in Epon. A saturated solution of uranyl acetate and lead citrate was used to contrast ultrathin sections, which were studied with a Philips EM 300 electronmicroscope.

RESULTS

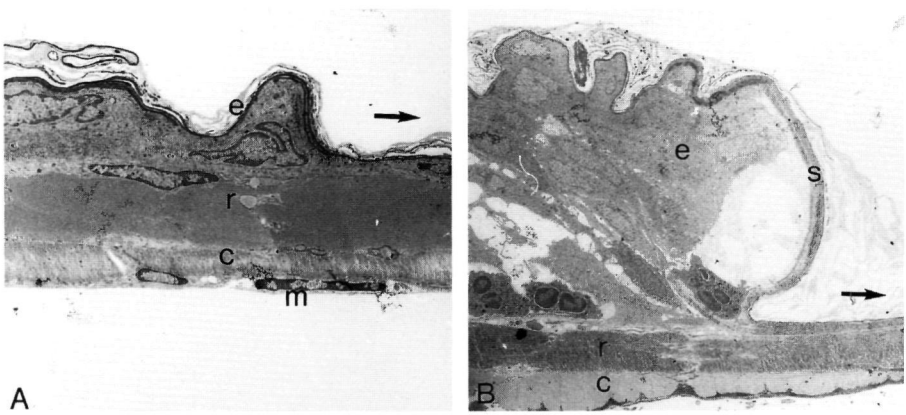

Figure 1. Electronmicrographs of traumatic pars tensa perforations after 7 hours (A) and 17 hours (B). Note piling up of epidermal cells (e) in A. At 17 hours the thickened epidermal front shows edema and is covered by an exudate. Note stratum corneum (s) lining the epidermal front and the denuded part of the dense fibrous layer. c: circular fibrous layer; r: radial fibrous layer; m: middle ear epithelium; arrow indicates site of perforation. Magnif. A x 1600, B x 1200.

Seven hours after perforation both the epidermal cells and middle ear epithelium were retracted from the perforation edges and piled up *(Figure 1A)*. The denuded part of the lamina propria, where some basal cells were left behind remained covered by the

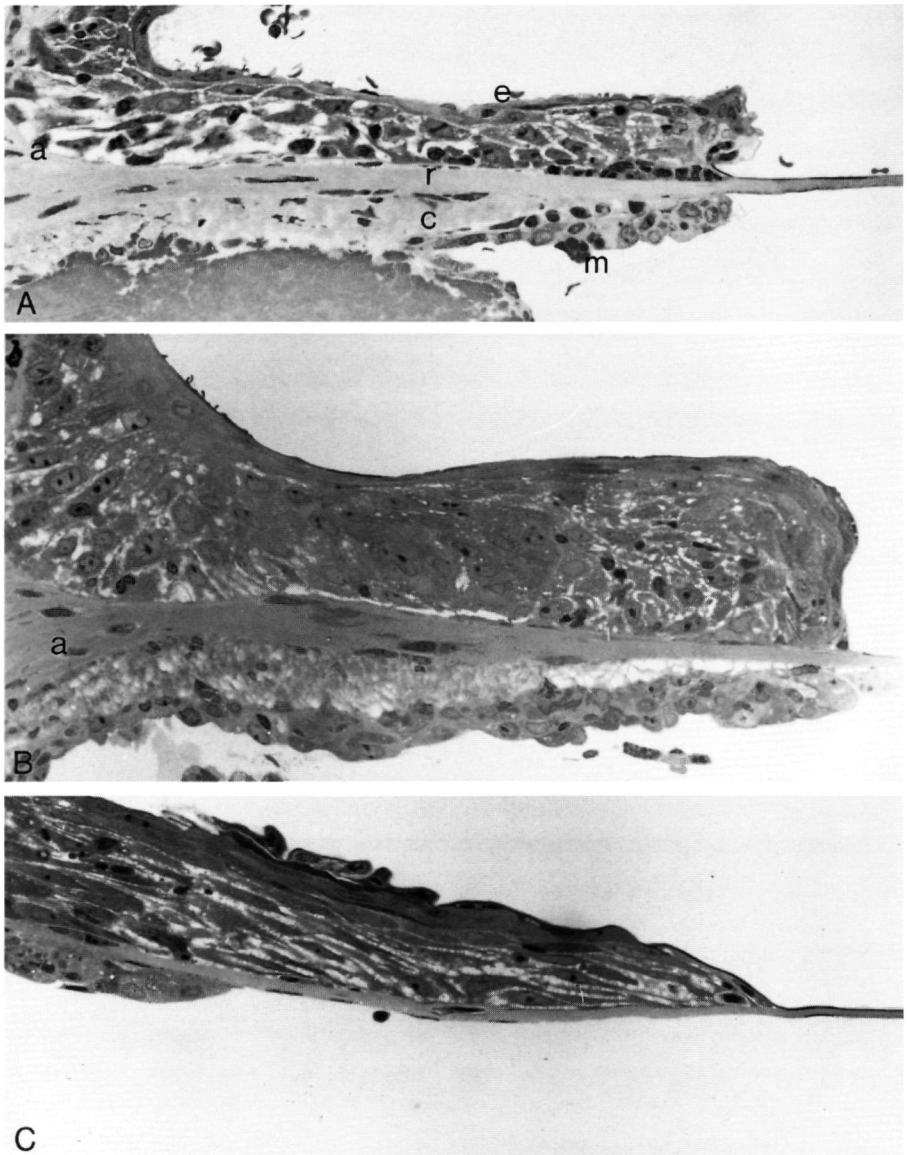

Figure 2. Lightmicrographs of traumatic pars tensa perforations after 1 (A) and 2 days (B,C). Note increased hyperplasia (A, B) and cornification and signs of migration in C. Micrographs in A and B originate from the annular area. Micrograph in C from area more distant from annulus. a: annulus; c: circular fibrous layer e: epidermis; m: middle ear epithelium; r: radial fibrous layer. Toluidin blue staining; magnif. A,B,C x 380.

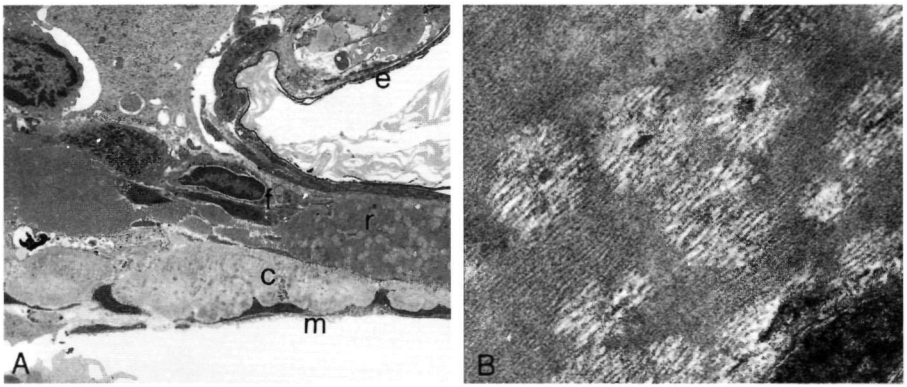

Figure 3. Electronmicrographs of epidermal front (e), 1 day after traumatic pars tensa perforation. Note inflammatory cells (f) in the dense fibrous layer, showing a moth-eaten appearance on the right side. B: higher magnification of moth-eaten fibrous tissue showing areas with tiny fibres and nucleation centre in the centre. c: circular fibrous layer; m: middle ear epithelium; r: radial fibrous layer. Magnif. A x 2000, B x 40.000.

stratum corneum. At 17 hours, the front of the retracted epidermal cells was covered by exudate containing polymorphonuclear leucocytes. The epidermal cells were hypertrophic and showed large intercellular spaces *(Figure 1B)*. Near the annulus and the handle of the malleus, dilated vessels were apparent. The basal cells on the denuded lamina propria were disintegrating.

At 1 day, epidermal hyperplasia was further increased and in addition the middle ear

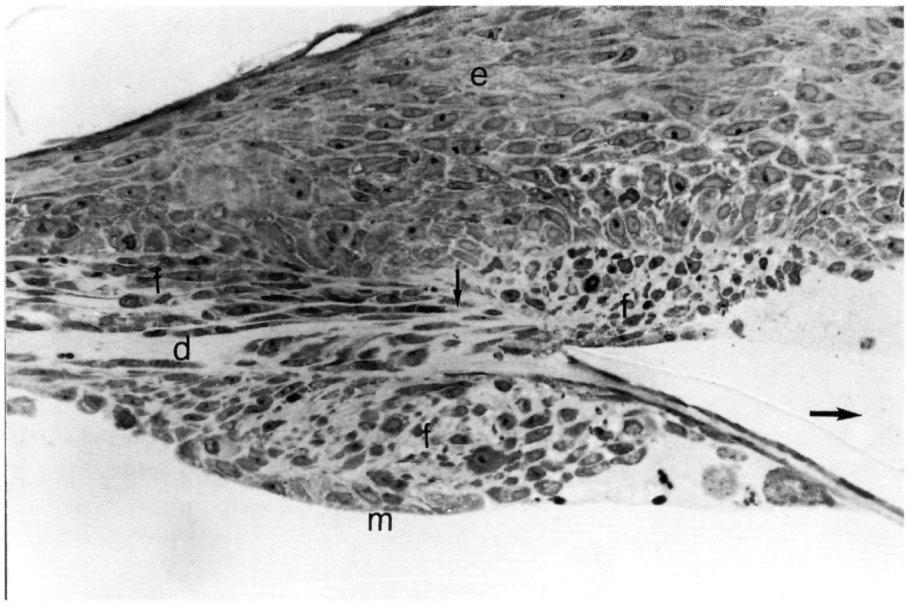

Figure 4. Lightmicrograph of epidermal front, 5 days after traumatic pars tensa perforation. The migrating epidermis (e) is largely thickened. Fibroblasts (f) which are present on both sides of the dense fibrous layer (d) locally penetrate this layer (small arrow). Large arrow indicates site of perforation. m: middle ear epithelium. Toluidin blue staining; magnif. x 380.

epithelium showed slight hyperplasia This reaction was most marked close to the annulus and the handle of the malleus and decreased with increasing distance from these structures Polymorphonuclear leucocytes were found scattered between the epithelial cells *(Figure 2A)* On LM sections no distinct alterations could be observed in the lamina propria However TEM revealed clear changes notably in the area below the front of the thickened epidermal edge and in front of the edge Both the circular and radial layers showed a moth eaten appearance exposing tiny fibres In the centre of these sites nucleation centres were visible *(Figure 3)* Towards the perforation edge the denuded avital lamina propria was still intact

At 2 days, the epidermal front showed stratification and starting cornification and moved towards the perforation edge along the persisting lamina propria Hypertrophy and hyperplasia were observed in the middle ear epithelium *(Figure 2B C)* Fibroblasts were present notably in the subepithelial areas, close to the annulus and the handle of the malleus At 5 days, the number of fibroblasts had strongly increased on both sides of the lamina propria at the front of the epidermis, replacing locally the disintegrating fibrous layer *(Figure 4)* At other sites, this layer showed numerous calcareous deposits *(Figure 5 A C)*, or appeared as a virtually unaffected avital structure *(Figure 5B)* At this stage, a distinct difference existed between the behaviour of the epidermis in the large marginal and the small central perforations In cases of marginal perforations, a large amount of fibrous tissue had been formed close to the annulus and the handle of the malleus The original lamina propria was often dislocated The epidermal cells migrated around the perforation edge, occasionally making contact with the middle ear epithelium *(Figure 6A)* In the small central perforations, epidermal cells migrated on top of the denuded lamina propria in the plane of the tympanic membrane towards the perforation edge *(Figure 4)* while the amount of fibrous tissue formed was less At 7-8 days, the epidermal cells had closed the gap in most of the small central perforations *(Figure 7A)*, shortly afterwards followed by fibroblasts and capillaries *(Figure 7B)* Simultaneously, the middle ear epithelium also restored continuity In those areas where the original dense fibrous layer persisted, this layer was embedded in newly formed connective tissue on both sides *(Figures 7B 8)* However, the collagen fibers appeared to be much thicker than the original fibres Closure of the marginal larger perforations occurred a few days later Epithelial closure was first observed at the bottom of a U-shaped dimple *(Figure 6B)*

During the next two weeks there was a gradual thinning of both the epidermal and connective tissue layer in the traumatised areas After about 4 weeks the membrane had obtained its final architecture The epidermal and middle ear epithelium did not differ from that outside the traumatised areas, but the middle layer was much thicker This condition did not change further throughout the observation period of one year In the middle layer, the site of the defects was marked by the often curled margins of the original avital dense fibrous layer *(Figure 9)* The gap was filled by connective tissue with a varying cellularity *(Figures 9 10A)* Away from the margins, the original dense fibrous layer was lined on both sides by newly formed connective tissue *(Figure 9)* This tissue and that filling the gap lacked the original circular and radial fibre arrangement, but at high magnification an alternating fibre orientation at different levels could be distinguished *(Figure 10B)* Lightmicroscopy showed numerous small spots at various sites of the persisting fibrous layer which reacted with

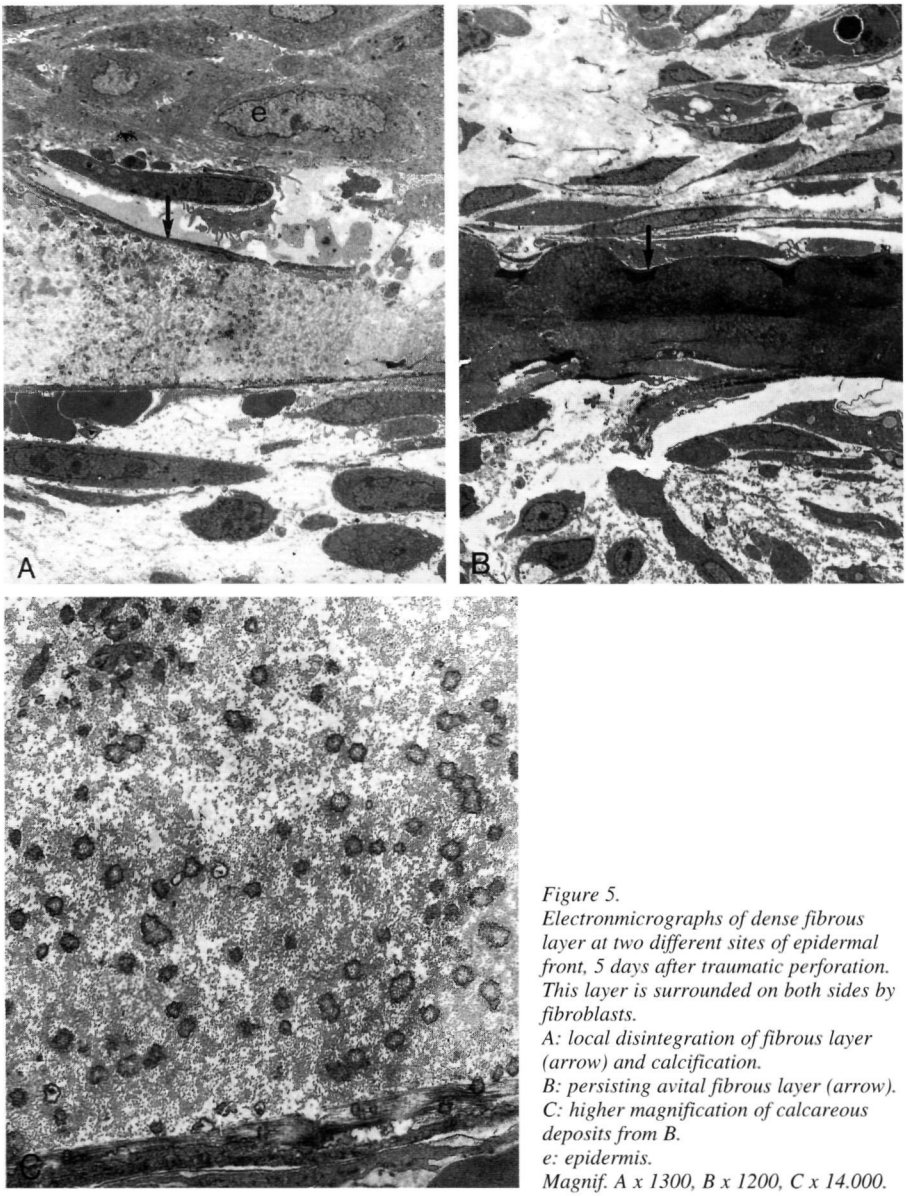

Figure 5.
Electronmicrographs of dense fibrous layer at two different sites of epidermal front, 5 days after traumatic perforation. This layer is surrounded on both sides by fibroblasts.
A: local disintegration of fibrous layer (arrow) and calcification.
B: persisting avital fibrous layer (arrow).
C: higher magnification of calcareous deposits from B.
e: epidermis.
Magnif. A x 1300, B x 1200, C x 14.000.

von Kossa's stain for calcium. Electronmicroscopy revealed numerous calcareous deposits in this structure, except at the margin *(Figure 11)*. These depositions were sometimes fused to larger plaques. Similar findings were also made in areas far distant from the traumatised area, where apparently no new fibrous tissue was formed and the dense fibrous tissue appeared vital *(Figure 12A)*. Calcareous deposits were also observed in the newly formed connective tissue *(Figure 12B)*. This condition did not change throughout the observation period up to 12 months.

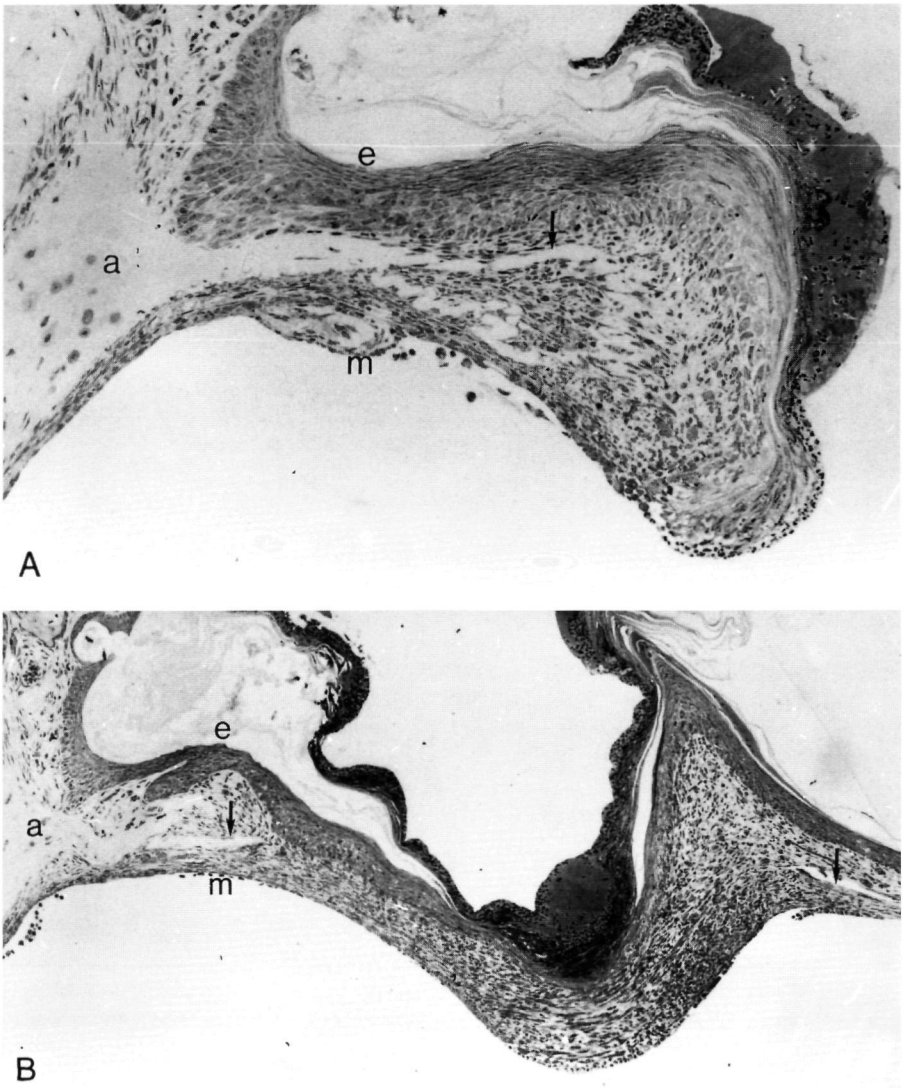

Figure 6. Lightmicrographs of large traumatic marginal perforation of the pars tensa, after 5 (A) and 11 (B) days. After 5 days the epidermal cells, supported by fibrous tissue, had migrated around the perforation edge. After 11 days epidermis and newly formed lamina propria had fused together at the bottom of a U-shaped dimple. a: annulus; e; epidermis; m: middle ear epithelium; arrow: persisting dense fibrous layer. Toluidin blue staining; magnif. A,B x 190.

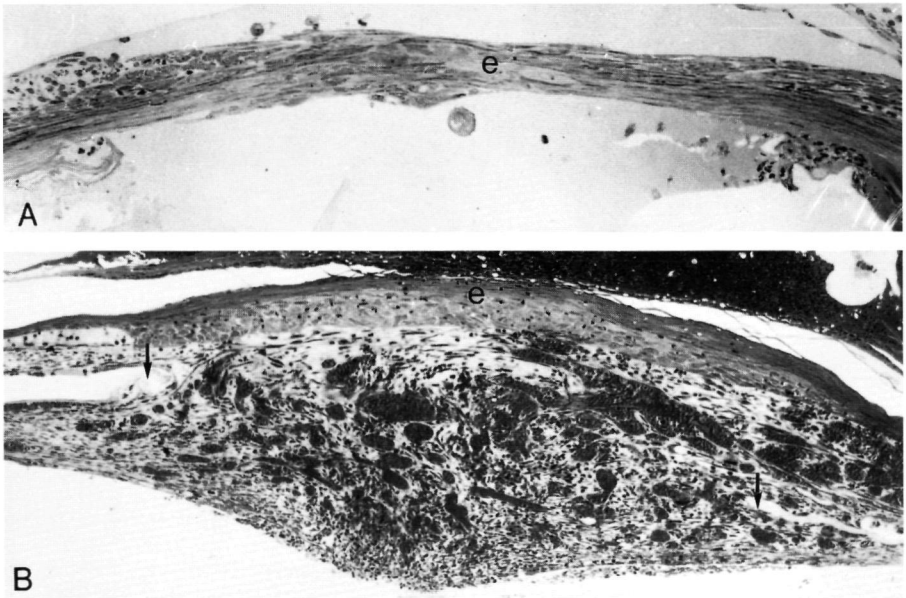

Figure 7.
Lightmicrographs of a small central perforation of the pars tensa after 7 (A) and 9 (B) days. A shows closure of the defect by epidermal cells. In B the gap is filled by fibroblasts and capillaries below the thickened epidermis. Arrows indicate margins of the perforation, marked by the persisting dense fibrous layer. e: epidermis. Toluidin blue staining; magnif. A,B x 190.

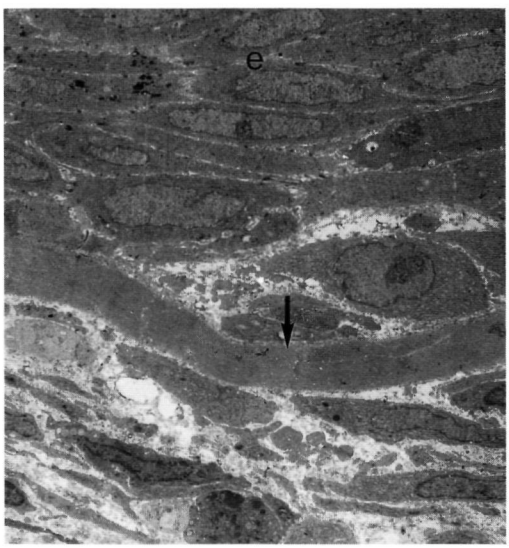

Figure 8.
Electronmicrograph of persisting dense fibrous layer (arrow) embedded in new lamina propria of nearly closed small pars tensa perforation after 8 days e: epidermis. Magnif. x 1200.

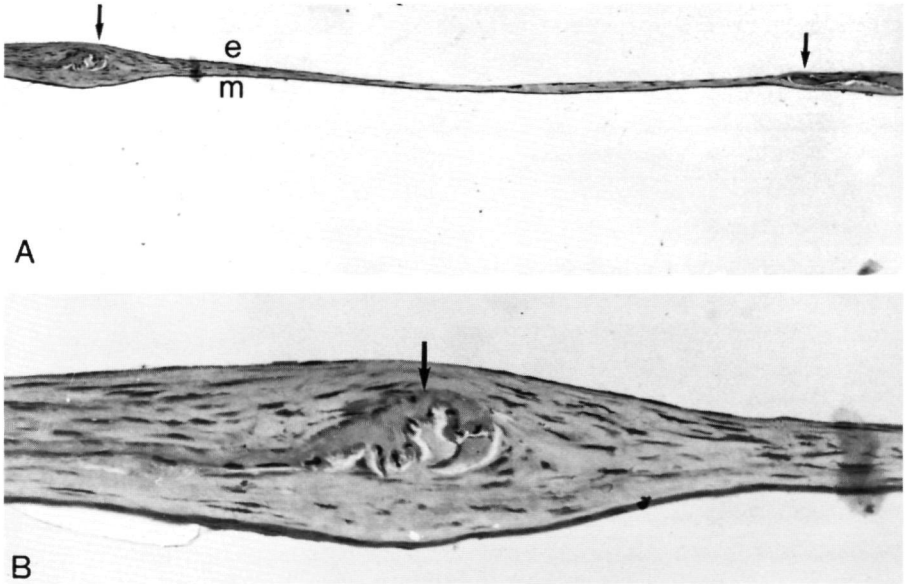

Figure 9. Light micrographs of healed small central traumatic perforation of pars tensa after 4 weeks. A: survey; B: higher magnification. The edges of the perforation are marked by the curled edges of the persisting dense fibrous layer (arrows) The healed part is composed of homogeneous connective tissue lined by a thin epithelial lining. At distance from the edge, the persisting fibrous layer is embedded in newly formed connective tissue. e: epidermis; m: middle ear epithelium. Toluidin blue staining; magnif. A x 80, B x 380.

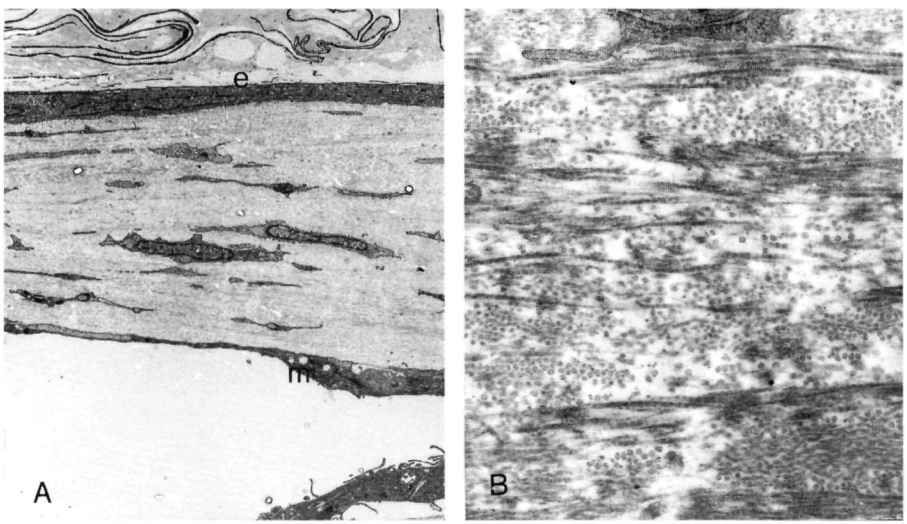

Figure 10. Electron micrographs of healed small central traumatic perforation of pars tensa after 4 weeks. A shows middle layer, composed of fairly homogeneous connective tissue. Higher magnification of middle layer in B shows alternating fibre orientation. e: epidermis; m: middle ear epithelium. Magnif. A x 1200, B x 15.000.

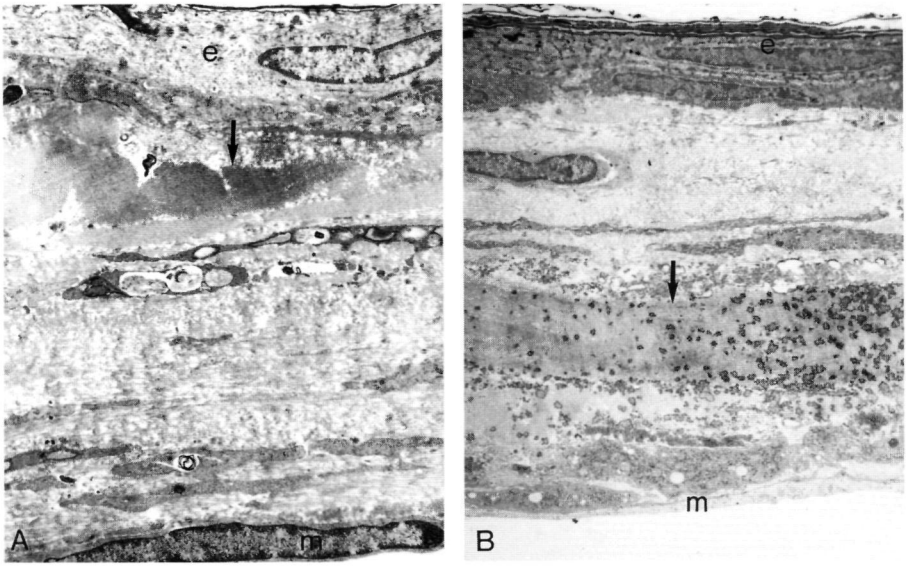

Figure 11. Electronmicrographs of healed traumatic tympanic perforation of pars tensa after 6 months. A shows margin of persisting dense avital fibrous layer (arrow). B shows calcareous deposits in this layer (arrow) at distance from the margin. e: epidermis; m: middle ear epithelium. Magnif. A x 5000, B x 2500.

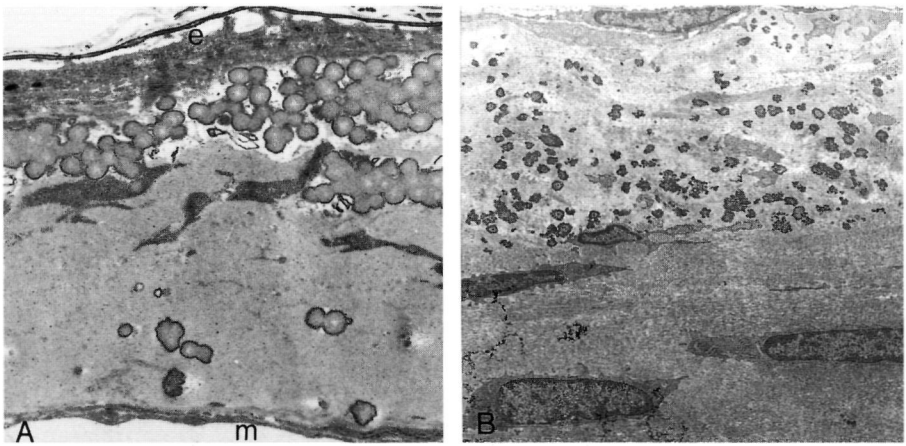

Figure 12. Electronmicrographs of pars tensa of tympanic membrane, 6 months after traumatic perforation. A shows calcareous deposits in dense vital fibrous tissue in area far distant from the site of perforation. B: shows calcareous deposits in newly formed connective tissue lateral from the persisting dense fibrous layer. e: epidermis; m: middle ear epithelium. Magnif. A x 2000, B x 7000.

Pars Flaccida

At 7 hours, the bare surface of the edematous lamina propria was covered by an exudate containing many polymorphonuclear leucocytes. At 17 hours, the amount of exudate had increased and inflammatory cells were also found in the lamina propria. The epidermis showed hypertrophy and hyperplasia *(Figure 13 A)*. At 1 day, epidermal

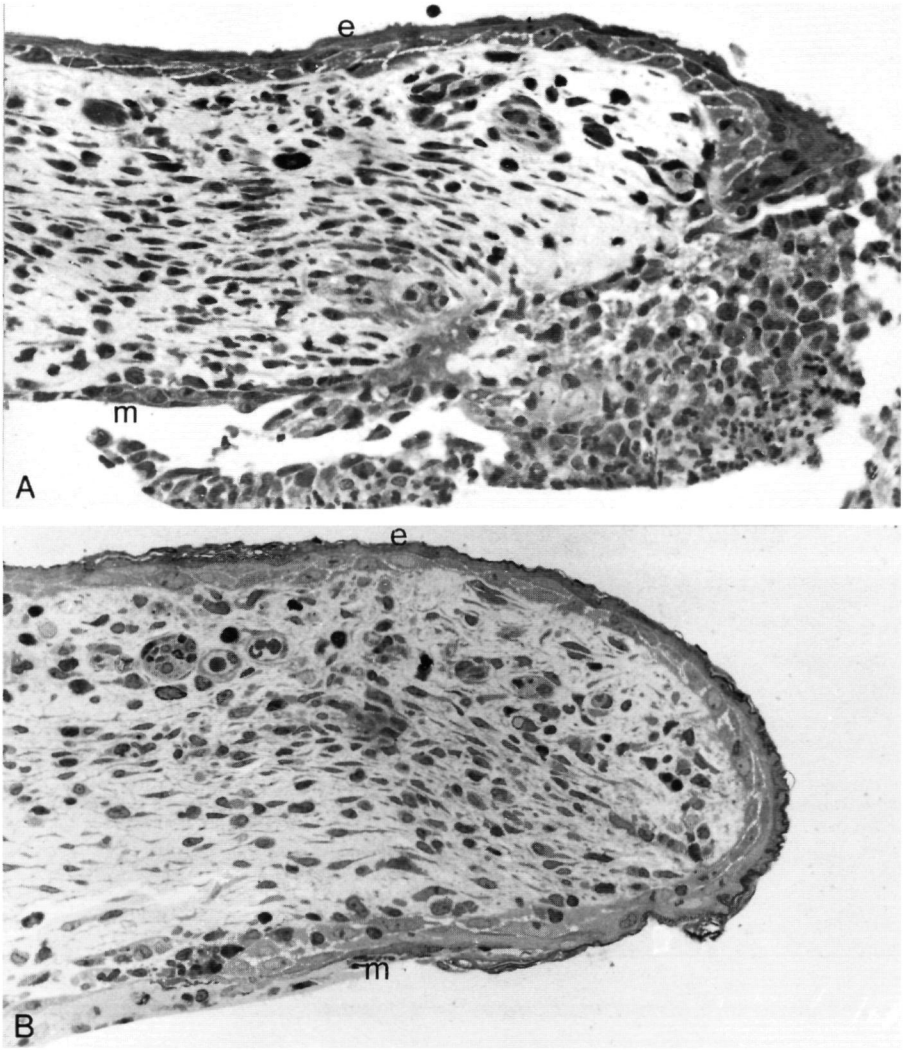

Figure 13. Lightmicrograph of traumatic perforation of pars flaccida after 17 hours (A) and 2 days (B). A shows migration of the hyperplastic epidermis (e) around the perforation edge. The wound surface is covered by a cellular exudate. In B the epidermis has covered the wound surface and has made contact with the middle ear epithelium (m). The lamina propria contains many capillaries and inflammatory cells. Toluidin blue staining; magnif. A,B x 380.

cells had migrated around the perforation edge. The middle ear epithelium was hypertrophic. The gap between both epithelia was still covered by an inflammatory exudate and numerous inflammatory cells were present in the lamina propria. At 2 days, the hyperplastic front of the epidermis had made contact with the hypertrophic middle ear epithelium in the major part of the wound edge. In the lamina propria the number of fibroblasts and capillaries had largely increased *(Figure 13 B)*. At 5 days, the perforation had closed in most cases by a gradual narrowing of the defect in a cir-

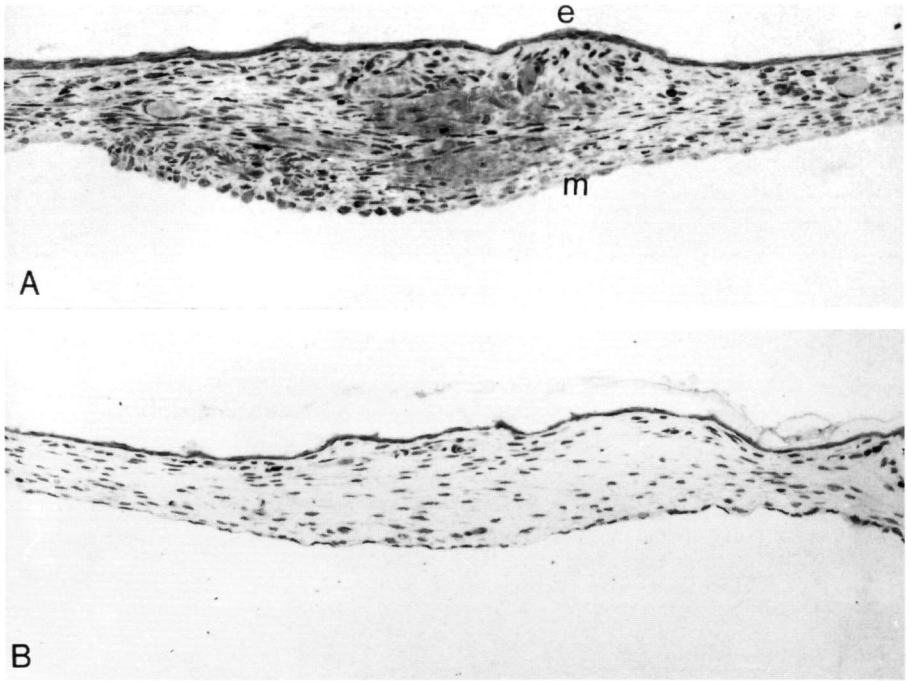

Figure 14. Lightmicrographs of healed traumatic perforations of the pars flaccida after 1 week (A) and 4 weeks (B). After 1 week, the healed area is composed of connective tissue with numerous fibroblasts, capillaries and inflammatory cells. After 4 weeks, the area of the defect is still locally thickened. e: epidermis; m: middle ear epithelium. Toluidin blue staining; magnif. A,B x 190.

cular manner. In the area of the former defect, many inflammatory cells and capillaries were present. Subsequently, the epidermis and lamina propria gradually grew thinner *(Figure 14A)*. At 4 weeks, the original defect was only marked by a locally thickened area *(Figure 14B)*, but after prolonged observation periods, no signs of the defect could be traced.

DISCUSSION

The present experimental study confirms earlier findings that traumatic tympanic membrane perforations are closed first by migrating epidermal cells, followed by the formation of the lamina propria and the middle ear epithelium.[9,10] However, the healing pattern of small central perforations appears different from larger marginal perforations. In small central perforations the epidermis closes without the support of a vital substrate as is the case in epidermal wound healing. In larger marginal perforations the epidermis makes contact with the middle ear epithelium and gradually closes the gap, supported by a layer of newly formed connective tissue. This different behaviour must be ascribed to the size and the site of the defect. In marginal perforations, the reaction will be much more abundant, because of the injury of the highly

vascularized areas near the annulus and the handle of the malleus In small central perforations the response will be minor, because this area only has a very poor vascular supply [9]

Irrespective of these different healing patterns both the epidermis and the middle ear epithelium regain their original features In contrast to this the architecture of the lamina propria is largely changed after healing from a traumatic defect This occurs not only in the area of the defect, but also distant from this area In the area of the defect the middle layer is composed of connective tissue with a varying number of fibroblasts This layer lacks the original circular and radial fibre distribution, but an alternating fibre distribution appeared to be formed, which gives the healed area more tensile strength At a distance from the perforation, the lamina propria is composed of the persisting original dense fibrous layer, separated from the epithelial lining by newly formed connective tissue Although the original fibrous layer is devitalised, it shows a remarkable resistance to resorption Even after 12 months there are no clear signs of active resorption of this tissue This layer only disappears at those sites where there is a severe fibroblast reaction, but at other sites it persists The circular and radial fibrous layers demonstrate a remarkable susceptibility to calcification This applies not only for the devitalised tissue but also in areas far distant from the defect where this layer is apparently vital In addition newly formed fibres revealed calcification

This observation is in agreement with similar events established in a previous study, where the lamina propria was injured by the induced inward retraction of the tympanic membrane after eustachian tube obstruction I [1](chapter II) This process can be assumed to represent a form of dystrophic calcification which can be observed in soft tissues at other sites of the body after injury [12 14]

The causal mechanism of dystrophic calcification is still unknown Deteriorated metabolism, necrotic changes and the nature of the collagen or the presence of special glycosaminoglycans have been suggested to be triggering factors [15 17] Although deterioration of the metabolism, due to interference with the blood supply and necrotic changes, apply for the present study, no reliable data are available on the chemical nature of the collagenous tissue involved However, the absence of calcareous deposits after perforating the pars flaccida might suggest the existence of a relationship between the structure and the chemical nature of this fibrous tissue and its proneness to calcification

This study demonstrates that perforation of the tympanic membrane results in persistent pathological changes of the lamina propria, while healing of the pars flaccida occurs without leaving any scars The observed calcification of the lamina propria can probably contribute to a better understanding of the extended calcified lesions observed after traumatic lesions induced by the insertion of tympanostomy tubes in cases of chronic otitis media [18 20]

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CHAPTER V

TYMPANOSCLEROSIS IN THE TYMPANIC MEMBRANE: INFLUENCE ON OUTCOME OF MYRINGOPLASTY

E W J Wielinga, A M H Derks and C W R J Cremers

Am J Otol 1995, 16 811-814

ABSTRACT

The effect of the presence of tympanosclerotic plaques in the tympanic membrane on the outcome of a myringoplasty procedure was evaluated in this retrospective study Long term results of a total number of 714 myringoplasties were analyzed and of these 555 were eligible for further study Three groups were studied separately ears without tympanosclerosis, ears with a plaque not exceeding one third of the tympanic membrane surface area and ears with a plaque involving more than one third of the surface area extending to the border of the perforation The latter group was subdivided into one in which during the operation the plaque was removed and one in which it was left in place Take rate percentages as well as post-operative hearing results were calculated for each separate group of ears Concerning graft take rate, it is concluded that the presence of tympanosclerosis in the tympanic membrane played no substantial part in the long-term outcome With regard to post-operative hearing results however, this study shows an average hearing gain of 6 dB when plaques exceeding one third of the tympanic membrane surface area were removed as part of the myringoplasty procedure

INTRODUCTION

Surgical closure of a tympanic membrane perforation is a well established procedure It may be either an operation in its own right or part of a more extensive procedure in chronic ear surgery

Many reports exist on short and long term results of myringoplasty Short term success rates are favourable almost without exception, varying from 81% to 96%,[1 9] while long-term results vary from 78 % to 92% [10 13] Factors that predispose to failure have been extensively studied in the past decades but so far no uniform agreement exists on a number of them

Some authors consider site of the perforation an important factor, stating either that anterior localization predisposes to an unfavorable take rate of the graft[6 11] or posteroinferior perforations carry a greater risk of reperforation [14] Others have found perforation site not to be significant [7 8 12 13]

Also, the size of the perforation often has been mentioned as a determining aspect Some reports indicate that large perforations are more prone to reperforation,[15 16] whereas other studies failed to show any influence of perforation size on take rate [7 8 12 13]

Other factors that have been debated include grafting techniques and graft material, condition of the middle ear during surgery, and the patient's age Concerning these, the majority of studies have indicated that better results are obtained with autologous grafting material, using the underlay technique, and that results are independant of the condition of the middle ear during surgery and the age of the patient [7 8 12 17]

It has been convincingly shown, however, that one of the main causes of failure is an immediate post-operative infection [16]

It has been argued that deposition of tympanosclerotic plaques in the perforated tympanic membrane might also complicate grafting and influence the final outcome [7 18 19]

To determine whether presence or removal of calcareous plaques in the tympanic membrane compromised graft take rate and post-operative hearing results, a retrospective analysis was performed by the authors of myringoplasties that took place between 1976 and 1986

PATIENTS AND METHODS

All patients who had a primary myringoplasty performed between January 1, 1976 and January 1, 1986, at the University Hospital Nijmegen were recalled and 692 of them (70 %) attended Twenty-two patients underwent bilateral operations, bringing the total to 714 myringoplasty procedures in 361 male and 353 female subjects, aged between 12 and 56 years

Table 1. Myringoplasty procedures performed

Total number of patients	985
Patients attending for long-term follow up	692
Total number of ears	714
Excluded for various reasons	159
chronic otitis	69
cholesteatoma	8
short follow-up	54
inadequate data	28
Ears selected for this study	555
Ears without tympanosclerosis	381
Ears with tympanosclerosis	174
Ears with small plaques (< 1/3 surface area)	112 out of 174
Ears with large plaques (> 1/3 surface area)	62 out of 174
Ears with tympanosclerosis left in place 1	38 out of 62
Ears with tympanosclerosis removed	24 out of 62

Inclusion criterion for ears to be accepted for this retrospective study was the presence of a tympanic membrane perforation with an otherwise healthy middle ear, that had not been operated on previously Exclusion criteria were the presence of chronic otitis, a cholesteatoma, a follow-up of less than 2 years and the absence of adequate data, which left 555 ears eligible for study (Table 1) Ears with tympanosclerosis in the tympanic membrane were subdivided into a group with a limited amount of tympanosclerosis (a plaque not exceeding 1/3 of the membrane surface) and a group with

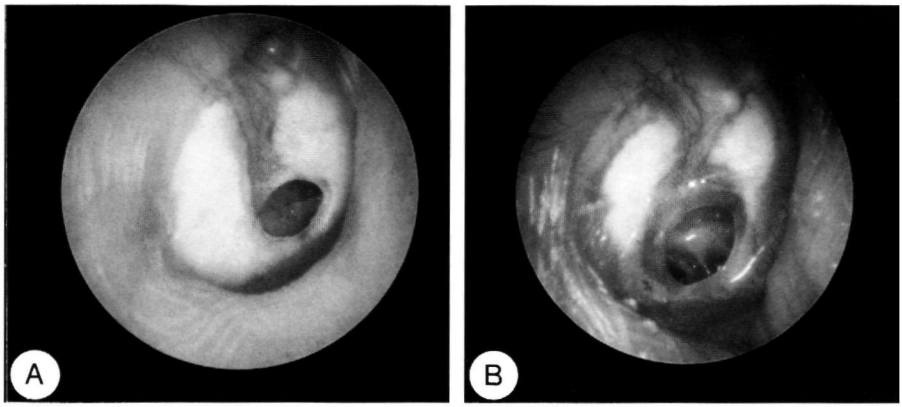

Figure 1 A. Plaque extending to and involving the perforation borders.
B. Solitary plaques of tympanosclerosis.

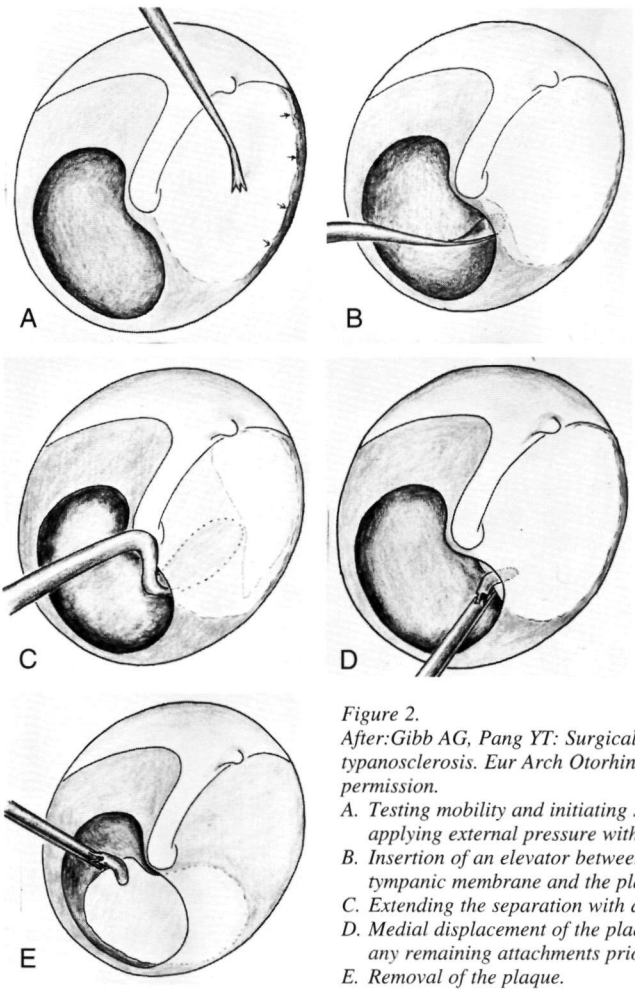

Figure 2.
After:Gibb AG, Pang YT: Surgical treatment of typanosclerosis. Eur Arch Otorhinolaryngol 1995;252:1-10. With permission.

A. Testing mobility and initiating separation of a fixed plaque by applying external pressure with a mobilizer.
B. Insertion of an elevator between the epithelial layer of the tympanic membrane and the plaque.
C. Extending the separation with an angled elevator.
D. Medial displacement of the plaque to ensure separation of any remaining attachments prior to removal.
E. Removal of the plaque.

extensive tympanosclerosis (a plaque involving more than 1/3 of the membrane and extending to the border of the perforation) *(Figure 1)* The latter group was further subdivided into two groups one in which the plaque was removed during surgery and one in which the plaque remained in place This required conclusive data concerning size and site of the tympanosclerotic plaque and whether it was removed during surgery These data were assessed on the basis of pre-operative drawings of the tympanic membrane and/or detailed surgical reports

Grafting took place in a similar manner in all cases Temporal muscle fascia was obtained and inserted onto the medial surface of the drum remnant Depending upon the site of the perforation (i e the visibility of the anterior perforation margin), either a postauricular or endaural approach was used As a rule, the ossicular chain was tested for its mobility in all cases *(Figure 2)*

The hearing result was evaluated by comparing the individual pre-operative audiogram and the one which was made at the last visit to the clinic The hearing thresholds were averaged over the frequency range 0 5 Hz 1 and 2 kHz Pure tone audiometry was performed using standard procedures and equipment The audiometer (Interacoustics AC 5 with TDH-39P headphones Telephonics, Huntington, New York) was calibrated according to ISO Standards 389

Tympanosclerotic fixation of the ossicular chain was found in 14 cases, all in ears with extensive tympanosclerosis, whereas interruption of the ossicular chain was found in four ears In determining the post-operative hearing results, these ears with coexisting ossicular pathology were excluded

The minimum follow-up period was 2 years with a maximum of 13 years, 6 months and a mean follow-up of 7 years 6 months The graft take rate of myringoplasties without tympanosclerosis and those with and without removal of the tympanosclerotic plaques from the tympanic membrane was calculated, and the results of these three groups were compared Also, the individual hearing result in these three groups was compared to determine whether removal of tympanosclerotic plaques influenced the outcome

RESULTS

The aetiology of the tympanic membrane perforations in this study was usually infection or prior ventilation tube insertion Most myringoplasties were performed on dry ears Perforations were usually located in the centre of the tympanic membrane The presence of an intact tympanic membrane at the last follow up visit was designated as a success The overall results of surgery are presented in Table 2

The total number of reperforations in 555 ears was 68 (12%), which amounts to an overall success rate of 88 % In the group without tympanosclerosis (n = 381), 41 reperforations were noted (11 %), that is, a take rate of 89 % Table 2 shows that in the tympanosclerosis group, including its subdivisions, take rates of between 83 % and 85 % were found There were no significant differences in take rate detected among any of the (sub) categories (chi square test, 5 % probability level)

Table 2. Take rate in ears with tympanosclerosis

	total number of ears with tympanosclerosis	moderate amount of tympanosclerosis	tympanosclerosis in border of perforations	
			tympanosclerosis	
			removed	not removed
myringoplasties	174	112	24	38
reperforations	27	17	4	6
take rate	147/174 (84%)	95/112 (85%)	20/24 (83%)	32/38 (84%)

Table 3 summarizes the hearing results of the different groups
The average air conduction gain of the non-tympanosclerotic group and the group with limited depositions was 11 dB and 10 dB, respectively. In the group with extensive tympanosclerosis, 18 ears had coexisting ossicular pathology They all were found in the group in which plaques were not removed Most of these (14) had tympanosclerotic fixation of the ossicular chain attributable to deposits that were often continuous with those in the tympanic membrane In these cases surgeons did not attempt removal
When large plaques had been removed, the mean individual change in air-bone (A-B) gap was 16 79 dB (SD 5 11), whereas when they were left in place, mean individual gain was 10 95 dB (SD 2 86) It should be noted that both of these groups had a similar A-B gap

Table 3. Hearing results of 555 myringoplasty procedures

		n	Pre-operative		Post-operative	
			AC	AB gap	AC	AB gap
No tympanosclerosis in TM		381	19	15	8	4
Tympano-sclerosis in TM	Small	112	20	16	10	6
	Large removed	24	26	22	10	6
	Large not removed	20*	26	22	16	12

* 18 ears excluded due to coexisting ossicular pathology
TM = tympanic membrane

before operation The difference (about 6 dB) in change in A-B gap was significant (Student's t-test, 42 degrees of freedom, $t = 4.55$, $p < .05$). A post-operative increase of bone conduction level was noted in three ears, not exceeding 15 dB. There were no dead ears.

DISCUSSION

Myringoplasty is considered to be a routine operation with generally good results A possible cause of failure is considered to be the presence of tympanosclerotic plaques in the tympanic membrane remnant. Tympanosclerotic plaques are situated in the lamina propria, and as bloodvessels are also situated in this layer, blood supply may be compromised which could have a negative influence on epithelial migration. Analysis of the results of this retrospective study showed no significant difference in the graft take rate between the tympanosclerotic and the non-tympanosclerotic groups. This is in agreement with a previous study by Gibb & Chang.[7]

When borders of the perforation are involved, one is tempted to excise deposits, but this can result in tears in the tympanic membrane remnant and a large perforation that is technically more difficult to close. This study showed that excision of such plaques did not give better results concerning take rate. Overall, graft take rates in all groups in this report with a follow-up as long as 13 years 6 months are similar to other reports in the literature. Hearing results in simple myringoplasty are, as a rule, favorable. The clinical importance of tympanosclerotic deposits in the tympanic membrane depends on their size. When only small deposits are present, hearing loss is usually insignificant.[20,21] In a study on children who had been previously treated with ventilation tubes for secretory otitis media, Tos & Poulsen found no difference in speech reception thresholds between ears with tympanosclerosis and ears with a healthy pars tensa.[22] Tos & Stangerup found a mean difference of maximally 1 dB at the frequencies 250, 1000 and 4000 Hz between thresholds of normal ears and ears with tympanosclerotic drums.[23]

A large plaque, however, may severely impair mobility of the tympanic membrane and result in a mild to moderate hearing loss.[24] It may be adherent either to the bony annulus or to the handle of the malleus or it may make contact with the promontory. When a large plaque involves the anterior half of the tympanic membrane, it may be fixed to the bony annulus in front and to the handle of the malleus behind, causing both immobility of the tympanic membrane and fixation of the ossicular chain. In this study this was the case in 14 ears.

Post-operative hearing results were as expected in the non-tympanosclerotic group and the group with small depositions with a mean A-B gap of 4 dB and 6 dB, respectively. This compares well with the hearing results in the group with a large plaque of tympanosclerosis that was removed during the operation, which resulted in a mean A-B gap of 5 dB. This is in accordance with the study of Emett & Shea,[25] although the numbers in that study were too small to justify any conclusions. In the group in which the plaques were not removed, however, the post-operative hearing result was worse with an average air-conduction of 16 dB and a mean A-B gap of 12 dB.

In conclusion this study shows that neither small nor large tympanosclerotic plaques in a perforated tympanic membrane compromise graft take rate. When large plaques are present, however, hearing will significantly benefit when depositions are removed.

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ACKNOWLEDGEMENT

The authors wish to acknowledge the help of P.L.M. Huygen, PhD, in the statistical analysis of the results.

CHAPTER VI

SUMMARY AND CONCLUSIONS
SAMENVATTING EN CONCLUSIES

SUMMARY AND CONCLUSIONS

Tympanosclerosis is a disease process which affects the mucosa of the middle ear cavity. Lesions consist of accumulations of abnormal fibrous tissue, showing areas of hyalinisation and calcification. The process involves mainly the lamina propria of the pars tensa of the tympanic membrane, but it may also affect the lamina propria of the mucosal lining of the bony wall of the middle ear and ossicular chain.
The exact pathogenesis of tympanosclerosis is not entirely understood, but in the course of many clinical studies, long-standing inflammatory processes in the middle ear and probably trauma have been assumed as important etiological factors.
Management of tympanosclerotic lesions is either surgical or conservative. Views on surgical procedures in cases of tympanosclerosis vary largely among surgeons.
In this thesis, first a critical analysis is presented, relating current notions on pathogenesis, clinical aspects and management of tympanosclerosis. Secondly, the pathogenesis of tympanosclerosis is studied using an experimental model, developed in our laboratory. Thirdly, long-term results are presented with regard to graft take rate as well as postoperative hearing of surgical procedures, performed in ears containing tympanosclerotic deposits in the tympanic membrane.

CHAPTER I contains an evaluation of current concepts regarding pathogenesis, clinical aspects, relation to other middle ear diseases and management of tympanosclerosis. In addition, a classification is proposed for tympanosclerosis occurring at different sites. This could facilitate comparison of surgical techniques and postoperative results.

In **CHAPTER II** an experimental model is presented for studying the development of tympanosclerosis. Obstruction of the eustachian tube in germfree rats with a subsequent sterile middle ear effusion and retraction of the eardrum was shown to result invariably in the development of tympanosclerotic lesions. The lesions were mainly confined to the lamina propria of the pars tensa of the tympanic membrane, but occasionally they were also observed in the mucosa, lining the bony wall of the middle ear after prolonged survival.
The development of the lesions was characterised by degeneration of fibrils and formation of abnormal fibrils and calcification of pre-existent and newly formed fibrous tissue. This process finally resulted in a largely thickened membrane, due to excessive formation of fibrous tissue with areas of calcification and hyalinisation. The histopathological features of these lesions were similar to those established in human specimens. It was concluded that the development of these lesions may be the direct result of sustained mechanical injury or deterioration of the blood supply resulting from underpressure in the middle ear.

In **CHAPTER III**, sterile and infected middle ear effusions were experimentally induced by eustachian tube obstruction in specific pathogen free rats and rats with an upper airway infection, respectively This model allowed for analysis of the effects of primary serous effusions, secondary re-aeration and primary and secondary infected effusions on the structure of the tympanic membrane. Serous effusions resulted in the

development of tympanosclerosis. Re-aeration of the middle ear resulted in the complete disappearance of calcareous deposits, but the accumulated abnormal fibrous tissue persisted. Secondary infected effusions did not affect pre-existing tympanosclerotic lesions. Tympanosclerosis never developed in the presence of primary infected effusions. These effusions resulted in a varying destruction of the lamina propria, related to the micro-organisms involved, followed by extensive fibrosis. According to these experimental observations it is discussed that the development of tympanosclerosis in chronic otitis media is more likely to be ascribed to mechanical injury than to the effect of inflammatory processes.

In **CHAPTER IV** the healing pattern of traumatically induced perforations in both pars tensa and pars flaccida is reported, with special emphasis on the lamina propria. The healing pattern appeared to depend on the site and the size of the peforation. The architecture of the epithelial lining regained its original structure, but the lamina propria was largely changed after completion of the healing process. It was composed of a homogeneous connective tissue, lacking the original dense fibrous structure, consisting of circularly and radially oriented fibres. Remnants of the original dense fibrous structure revealed a remarkable resistance to resorption. They persisted throughout an observation period of 1 year. Moreover, this structure and also the newly formed connective tissue showed a high susceptibility to calcification, even at distance from the perforated area. Calcification was assumed to represent a form of dystrophic calcification. In contrast to the pars tensa, no structural changes were established in the pars flaccida after healing.

Summarizing, the results of these experimental studies demostrate that both serous and infective middle ear effusions result in irreversible changes of the lamina propria of the pars tensa. Mechanical damage to the pars tensa, rather than the effect of inflammatory processes appears to be a crucial factor in the pathogenesis of tympanosclerosis.

In **CHAPTER V** results are presented of myringoplasties, performed on tympanic membranes containing tympanosclerosis of varying severity and situated at different locations. The study reports on a total of 555 myringoplasties with a follow up of more than 13 years.
No significant difference was found in the graft take rate between the group with tympanosclerosis of the eardrum and that without. Moreover, excision of plaques extending to the perforation borders had no influence on the final outcome concerning take rate. In cases with large plaques comprising over 1/3 of the surface area, removal showed better hearing than when they were left in place. However, removal of tympanosclerotic plaques from remnants of the eardrum as part of a surgical procedure presents hazard of tearing of the tympanic membrane and requires a careful surgical technique.

SAMENVATTING EN CONCLUSIES

Tympanosclerose is een aandoening van de lamina propria van het middenoorslijmvlies. De laesies bestaan uit ophopingen van abnormaal fibreus weefsel met gebieden die hyalinisatie en calcificatie vertonen. Ze komen voornamelijk voor in de pars tensa van het trommelvlies, maar ook de slijmvliesbekleding van de middenoorholte en van de gehoorbeenketen kan aangetast zijn.
De exacte pathogenese is nog steeds niet opgehelderd, maar uit een groot aantal klinische studies is duidelijk naar voren gekomen dat chronische otitis media en trauma van het trommelvlies belangrijke etiologische factoren zijn.
Tympanosclerotische laesies worden chirurgisch of conservatief behandeld, maar over de te volgen chirurgische procedure bestaat geen eenstemmigheid.
Het doel van dit onderzoek was om, naast een evaluatie van de verschillende theorieen over de pathogenese de klinische aspecten en de behandeling van tympanosclerose, meer inzicht te krijgen in het ontstaan van deze aandoening met behulp van een, in ons laboratorium ontwikkeld, diermodel.

Hoofdstuk I bevat een kritische analyse van de huidige denkbeelden met betrekking tot de pathogenese, de klinische aspecten, de relatie met andere middenooraandoeningen en de behandeling van tympanosclerose. Er wordt een classificatie voorgesteld voor tympanosclerose, wanneer de laesies op verschillende plaatsen voorkomen om de vergelijking van chirurgische en postoperatieve resultaten te vergemakkelijken.

In **Hoofdstuk II** wordt een experimenteel onderzoek bij kiemvrije ratten beschreven. Wanneer bij deze dieren een steriele middenooreffusie werd geinduceerd, door afsluiting van de buis van Eustachius, ontwikkelden zich steeds tympanosclerotische laesies in de pars tensa van het trommelvlies. Na langere obstructieperioden werden deze laesies ook incidenteel in het slijmvlies van de middenoorholte aangetroffen. Het ontstaan van deze laesies werd gekenmerkt door degeneratie van collagene vezels, de vorming van abnormale vezels en calcificatie van pre-existent en nieuw gevormd fibreus weefsel. Dit proces resulteerde uiteindelijk in een sterk verdikte pars tensa als gevolg van excessieve bindweefselvorming met gebieden die hyalinisatie en calcificatie vertoonden. De histopathologische kenmerken van deze laesies waren identiek aan die bij humane tympanosclerose. Er werd geconcludeerd dat deze laesies vermoedelijk het gevolg zijn van beschadiging van de lamina propria en/of van een gecompromitteerde bloedvoorziening als gevolg van de onderdruk, die in het middenoor wordt geinduceerd door afsluiting van de buis van Eustachius.

In de experimentele studie in **Hoofdstuk III** werden middenooreffusies geinduceerd door afsluiting van de tuba Eustachii bij specifiek pathogeen vrije ratten en ratten met een infectie van de bovenste luchtwegen. Met dit model was het niet alleen mogelijk om de invloed te bestuderen van primair sereuze (steriele) middenooreffusies en van primair geinfecteerde effusies op de structuur van het trommelvlies, maar ook die van secundair geinfecteerde effusies en van herbeluchting van het middenoor. Dit vormt een goede imitatie van de symptomatologie bij chronische otitis media. Sereuze effusies hadden steeds tympanosclerose tot gevolg. Secundaire beluchting resulteerde in

het volledig verdwijnen van de calciumdeposities uit de tympanosclerotische laesies, maar het abnormale fibreuze weefsel bleef bestaan Secundaire infectie van sereuze effusies had geen duidelijke invloed op de gevormde tympanosclerotische laesies Primair geïnfecteerde effusies hadden een variabele destructie van de lamina propria van de pars tensa tot gevolg Deze werd gevolgd door uitgebreide fibrose, maar er ontstonden geen tympanosclerotische laesies Op grond van deze waarnemingen werd gepostuleerd, dat het ontstaan van tympanosclerose bij chronische otitis media moet worden toegeschreven aan mechanische beschadiging van de lamina propria van het trommelvlies als gevolg van de onderdruk in het middenoor en niet aan ontstekingsprocessen

In **Hoofdstuk IV** wordt het genezingsproces beschreven van traumatische trommelvliesperforaties bij normale ratten Bij dit onderzoek werd speciale aandacht besteed aan de lamina propria Het genezingspatroon bleek sterk afhankelijk te zijn van de plaats en de afmeting van de perforaties Na genezing verschilde de epitheliale bekleding van het trommelvlies niet van het oorspronkelijke epitheel, maar de structuur van de lamina propria van de pars tensa was ingrijpend gewijzigd In het gebied van het defect bestond deze uit homogeen bindweefsel en de oorspronkelijke rangschikking in een dichte laag circulaire en een laag radiaire vezels was afwezig In de directe omgeving van het defect was de oude dichte fibreuze laag, die omgeven was door nieuw gevormd bindweefsel, avitaal, maar ze vertoonde geen duidelijke tekenen van resorptie gedurende de observatieperiode van 1 jaar In deze laag, maar ook in een deel van het nieuw gevormde bindweefsel, trad calcificatie op Dit proces vertoonde sterke overeenkomst met dystrophische calcificatie in zachte weefsels elders in het lichaam
In de pars flaccida werden, na perforatie, in tegenstelling tot de pars tensa, geen blijvende structurele veranderingen waargenomen

Samengevat tonen deze dierexperimentele studies aan, dat de inductie van zowel steriele als van geïnfecteerde middenooreffusies leidt tot irreversibele, maar onderling verschillende, veranderingen in de lamina propria van de pars tensa Het ontstaan van tympanosclerotische laesies blijkt sterk gerelateerd te zijn aan mechanische beschadiging van het trommelvlies en niet aan chronische ontstekingsprocessen

In **Hoofdstuk V** worden de resultaten geanalyseerd van een groot aantal myringoplastieken bij trommelvliezen met verschillende graden van tympanosclerose De studie omvatte 555 myringoplastieken, die gemiddeld meer dan 13 jaar gevolgd werden Het percentage gesloten trommelvliezen bleek niet significant te verschillen bij trommelvliezen met en zonder tympanosclerose Verder kwam naar voren, dat het verwijderen van plaques, die zich uitstrekten tot aan de perforatierand, geen invloed had op het aantal gesloten perforaties De verwijdering van plaques, die meer dan 1/3 van het trommelvlies besloegen, resulteerde in een beter gehoor dan wanneer deze in situ werden gelaten Hierbij dient opgemerkt te worden dat de verwijdering van tympanosclerotische plaques een zorgvuldige chirurgische techniek vereist, omdat het trommelvlies hierbij gemakkelijk kan scheuren

DANKWOORD

Graag bedank ik allen die aan het tot stand komen van dit proefschrift hebben bijgedragen
In het bijzonder dank ik dr W Kuijpers, prof dr P van den Broek en dr P H.K. Jap, die altijd bereid waren stukken kritisch door te lezen en suggesties te doen voor verbetering
De medewerkers van het laboratorium KNO, te weten Els Camps, Edith Tonnaer en Theo Peters dank ik voor hun onontbeerlijke hulp en niet aflatend optimisme.
Voorts wil ik de medewerkers van het Centraal Dierenlaboratorium danken voor hun ondersteuning bij de dierproeven.

CURRICULUM VITAE

De auteur van dit proefschrift werd geboren op 24 november 1954 te Rotterdam Hij studeerde van 1977 tot 1984 geneeskunde aan de Rijksuniversiteit te Leiden In 1985 was hij assistent chirurgie (AGNIO) in het Rode Kruis Ziekenhuis te Den Haag Later in dat jaar begon hij de opleiding tot Keel-, Neus- en Oorarts in het Academisch Ziekenhuis Nijmegen (opleider prof dr P van den Broek) Een deel van die opleiding heeft de auteur volbracht in het Royal Victoria Hospital te Belfast (Noord-Ierland), waar hij van 1988 tot 1989 werkte als senior registrar onder leiding van mr G D L Smyth In 1989 voltooide hij de opleiding Sindsdien is hij als staflid verbonden aan de afdeling Keel-, Neus- en Oorheelkunde van het Academisch Ziekenhuis Nijmegen

STELLINGEN

behorende bij het proefschrift:

Tympanosclerosis. An experimental and clinical study.

1 Tympanosclerosis is the least amenable middle ear disease to confront the otologist (G D L Smyth)

2 Langdurige retractie van het trommelvlies bij otitis media met effusie is een cruciale factor bij de pathogenese van tympanosclerose van de pars tensa (Dit proefschrift)

3 De algemeen aanvaarde theorie dat tympanosclerose het gevolg is van infectieuze middenoorontstekingen wordt niet door dierexperimenteel onderzoek ondersteund (Dit proefschrift)

4 Het herstel van trommelvliesdefecten wordt niet beïnvloed door de aanwezigheid van tympanosclerotische plaques (Dit proefschrift)

5 "In der Beschränkung zeigt sich der Meister" een goede dokter weet wat hij niet kan

6 Gerichte regionale augmentatie met autoloog weefsel is de belangrijkste steunpilaar van de hedendaagse rhinoplastiek

7 Polyposis nasi is een locale uiting van een constitutionele aandoening

8 Bij de chirurgische behandeling van het juveniele neus keelfibroom moet altijd de fossa pterygopalatina geëxploreerd worden

9 Bij de beslissing tot gehoorsverbeterende chirurgie verdient hanteren van de "Belfast rule of thumb" de voorkeur boven de Glasgow benefit plot

10 De basisschool dient voor het verwerven, de middelbare school voor het verwerken en de universiteit voor het verwerpen van kennis

11 Overdreven aandacht voor het aantal publicaties dient slechts het persoonlijk en niet het wetenschappelijk belang

12 Stellingen worden steeds minder op hun wetenschappelijk dan op hun NRC gehalte getoetst

E W J Wielinga